Chitosan based polymer/bioglass composites for tissue engineering applications

Djurdja Vukajlovic*, Julie Parker, Oana Bretcanu and Katarina Novakovic

School of Engineering, Newcastle University, UK

*Corresponding author: D.Vukajlovic2@newcastle.ac.uk

Keywords: Composite scaffolds; polymer/bioglass; hybrids, chitosan materials; genipin cross-linking

Abstract

Composite scaffolds formed from polymers and bioglasses have been widely explored for applications in regenerative medicine as they have suitable organic/inorganic structures and properties similar to human hard tissue. Yet, these materials have only been used for non-load-bearing or low load-bearing purposes as they have limited mechanical strength while research is focused on improving their properties. One method of improving mechanical strength is by covalently bonding the organic and inorganic phases. This has been successfully achieved in *Class II hybrids* which have covalent bonding between polymers and bioglasses. As well as improving mechanical strength, the chemical connection of the two phases results in simultaneous degradation. The currently available composite scaffolds use collagen for the polymer phase which can cause allergic reactions and transmit pathogens. An alternative natural polymer is chitosan which has been used to create scaffolds with bioglass avoiding the issues arising from collagen. Additionally, using cross-linking agents has been shown to strengthen chitosan hydrogels improving their mechanical properties. A promising natural cross-linker is genipin which has lower toxicity than other cross-linking agents while producing hydrogels with improved mechanical properties compared to pure chitosan. In this paper we offer an overview of requirements, structures and currently available composite scaffolds for tissue engineering applications. We discuss the limitations of the currently available materials and consider the potential of covalently bonded hybrids particularly in relation to chitosan-based materials and the added benefits of genipin cross-linking.

1. Introduction - The need for synthetic bone replacements and application of polymer/ceramic materials

Tissue engineering is a promising field of research which aims to replace and repair damaged or diseased tissues and organs with substitutes made of synthetic or natural compounds. It involves prostheses and parts with the ability to regenerate tissues, as well as vehicles for the delivery of drugs, cells or biomolecules, and the coating of non-biological apparatus (e.g. stents) with bioactive materials to enable interaction with cells [1]. According to data from 2016, the biomaterial market was

estimated to be $70.90 billion and is expected to reach $149.17 billion by 2021 [2]. Due to injury, disease, surgical procedures, increasing life span and a rising population, orthopaedic implants are in particular demand [3].

After blood, bone transplantation is the second most common tissue transplantation with over 2 million bone graft operations taking place every year worldwide [4, 5]. Autografts, grafting tissue from the same donor from one bone tissue to another, continue to be the gold standard. They are used in about 60% of all bone graft procedures due to their ability to promote bone formation and induce bone tissue growth without causing an immune response [5]. Other options include allografts (tissue from the same species but not genetically identical), used in about 35% of bone graft procedures, and xenografts (when the donor is another species) [5]. However, certain issues associated with these natural materials can arise: a lack of supply of suitable tissue for large defects when using autografts; donor site complications (most commonly persistent pain) and a negative immune response of the patient that can occur when using allografts or xenografts [3, 5, 6]. For these reasons, synthetic materials have been widely investigated as alternatives [1, 3, 6].

In order to replace natural tissue, synthetic materials need to have similar properties to native tissue [7]. Since its beginning 30 years ago, tissue engineering has developed greatly and there are numerous commercially available synthetic bone graft substitutes [5, 8-13]. These are composites of mainly collagen and calcium phosphate, mostly used for dental applications and for repairing joints and broken bones [5, 8, 9, 11-13]. These composites have a composition similar to natural bone tissue (composed of an inorganic phase (hydroxyapatite) and an organic phase (collagen)). They overcome the problems linked to auto- and allografts mentioned previously, and have the potential to achieve enhanced mechanical properties combining the strength of ceramics with the flexibility of polymers [5]. Furthermore, producing composites with natural polymers has the added advantages of biocompatibility and the ability to stimulate cells, enhancing adhesion and differentiation. Natural polymers such as gelatin and chitosan are mainly degraded by enzymes. In chitosan, the enzymes gradually hydrolyse the bonds between the glucosamine and N-acetyl-glucosamine units present in the chitosan structure [14]. This is a promising feature compared to synthetic polymers like polyesters (US Food and Drug Administration (FDA) approved polymers widely used for sutures, drug delivery, vascular grafts, skin replacement, dental and orthopaedic implants and cartilage repair) which degrade rapidly once degradation begins [4, 15]. The combinations of ceramics and natural polymer materials reported so far have been limited to non-load-bearing and low load-bearing applications. However, due to their properties, such as bone-like architecture, ability to stimulate and enable new

bone and blood vessel formation as well as encouraging results in clinical applications, ceramic/polymer composites continue to be studied [5, 7-9, 16-20].

2. Scaffolds and their properties

One way to employ ceramic/polymer composite materials is to design them into scaffolds. Scaffold materials are porous and degradable structures that should provide mechanical support to bone defects, and allow cells to proliferate and differentiate [17, 21]. In order to be successful, scaffolds must fulfil numerous requirements:

1) Biocompatibility – this essential property for biomaterials is defined as the ability of a material to support normal cellular activity without causing local or systemic damage to the surrounding living tissue [17]. Ideally, the scaffold should also be osteoconductive (allow bone growth on its surface and in the pores) and osteoinductive (stimulate non-differentiated cells to develop into bone-forming cells) [4, 5, 17, 22]. The porous structure of the scaffolds should allow formation of new blood vessels [4, 17].
2) Bioactivity – this represents the ability of the scaffold's surface to form an apatite layer (bone-like layer of hydroxyapatite) through which the scaffold will bond to the native bone tissue when implanted into the body. A test method first developed by Kokubo for bioactive glass ceramics employs a simulated body fluid (SBF) which has a composition similar to human blood plasma [23]. This test can be used to detect apatite formation on the surface of a composite material and predict apatite formation *in vivo* (ISO 23317:2014).
3) Mechanical properties – it is important that the mechanical properties of the synthetic material match those of the surrounding tissue, along with the load transfer between the implant and the tissue [4, 5, 17]. Properties of cancellous and cortical bone are given in Table 1. Load-bearing materials should have mechanical properties within the range of properties of cortical bone (low load-bearing materials exhibit mechanical properties near the lower limit of cortical bone and high load-bearing materials close to the upper limit of the cortical bone range) [24].

Table 1. Mechanical properties of cancellous and cortical bone and synthetic scaffolds [5, 17, 25-27]

Property	Cancellous bone	Cortical bone	PLGA/45S5 Bioglass scaffold	Chitosan/bioglass scaffold
Compressive strength	2 – 20 MPa	100 - 200 MPa	0.42 MPa	7.68 MPa
Compressive modulus	0.1- 2 GPa	15 - 20 GPa	51 MPa	0.46 GPa
Tensile strength	10 - 20 MPa	90 – 130 MPa	Not reported	3.11 MPa
Young's modulus	0.1- 4.5 GPa	17 – 24 GPa	Not reported	0.196 GPa
Fracture toughness	0.1-0.8 MPa $m^{1/2}$	2-12 MPa $m^{1/2}$	Not reported	0.24 MPa $m^{1/2}$

So far, synthetic scaffolds are not yet achieving the higher end of the desired properties, which is why their applications have been limited to low load-bearing conditions such as bone fillers and maxillofacial reconstructive procedures [5].

4) Porosity - scaffolds with pore sizes of 20-1500 μm have been reported in bone tissue engineering [28]. According to *in vitro* and *in vivo* studies, scaffolds are required to possess open interconnected pores with at least 100 μm in diameter in order to enable successful transport of food and oxygen for the cells and to allow removal of waste products [5, 17, 28-30]. *In vivo* studies showed that only pores larger than 100 μm enable healthy mineralized bone formation, while smaller size pores (75 – 100 μm) lead to the formation of unmineralized bone tissue which causes disease and disorders (e.g. pain, muscle weakness and fracturing of the bone) [30]. At the same time, when the pores had diameters in the region of 10 - 75 μm, formation of fibrous (scar) tissue, was reported [30]. Fibrous tissue only connects with the surrounding tissue but does not possess mechanical and other characteristics of bone tissue, which also manifests as disease and disorder. Furthermore, several reports suggest that the optimum pore size is 200 – 350 μm, and that both micro- and macroporosity is needed to enhance bone ingrowth [17, 28, 30]. Although greater porosity, is beneficial for cells to proliferate and differentiate it reduces the mechanical strength of the scaffold [30]. It is worth noting that healthy cancellous has a porosity of 50-90% with pore sizes of 300 - 600 μm, and cortical bone has a porosity of 3-12% with pore sizes of 10 – 50 μm in diameter [30, 31]. These values, as well as the strength and modulus of the bone, vary depending on race, age and sex [5, 30-32]. Vitoss™ (Orthovita), a collagen I/β-tricalcium phosphate composite scaffold used clinically, has 90% porosity with pores ranging from 1 to 900 μm which encompasses the range of healthy cancellous bone [12].

Despite the necessity for macroporosity, it has been demonstrated that micropores also play an important role for osteogenesis [33]. The apatite layer, which forms on the surface of bioactive implants after immersion in a simulated environment or *in vivo* is important for bone growth because

it initiates cell adhesion, proliferation and differentiation [34]. This apatite layer can also serve as a conductor for bone growth [34]. Since micropores (< 20 μm) enlarge the surface area, it is easier for the apatite layer to be formed (due to easier ion exchange by dissolution and precipitation processes), and is easier for proteins to attach to the surface of the material, leading to enhanced cell activity [29, 33]. It is worth reiterating that macroporosity has a bigger role in affecting the mechanical properties of scaffolds than microporosity [29]. The reason for this is that structures with large pores are only connected via necks/bridges which offer weaker support than denser structures. For example, in 45S5 Bioglass scaffolds with 60% porosity reducing the pore size from 700 μm to 400 μm increased compressive strength from 3.5 to 6.7 MPa [35].

The geometry of the pores present in the scaffold is shown to dictate the pattern of bone formation: discontinuous bone ingrowth was found in specimens containing controlled, network-structured pores, while continuous growth was seen in scaffolds with randomly distributed pores. It was concluded that discontinuous bone ingrowth tends to result in faster filling of the scaffold when compared to continuous growth; however, the overall amount of bone formation remained unaffected [36]. Furthermore, when the samples contained microporous walls as well as a systematic, organized architecture, both types of bone ingrowth were detected (continuous and discontinuous). These findings suggest that by controlling the pore size of scaffolds, and most importantly pore architecture, it is possible to direct the pattern, discontinuous or continuous, as well as time of bone growth [36].

5) Biodegradability - scaffolds are expected to degrade over time to make room for the new tissue to grow. After implantation, scaffolds should have a similar strength to the host tissue and degrade over time with a controlled rate, depending on the application [4, 5, 17]. Reported degradation times vary from 3 to 6 months for scaffolds used in cranio-maxillofacial (skull, face, jaws, mouth) procedures to approximately 9 months or more for those used in spinal fusion [17]. For example, RegenOss® (JRI Orthopaedics), a clinically used collagen l/hydroxyapatite scaffold, degrades between 6 and 12 months after implantation [12]. Ideally, products formed by degradation of the implanted material should be non-toxic.
6) Porosity and degradation rate interplay – these two factors need to be tuned, that is if the degradation rate of the material is high, initial porosity needs to be low otherwise the scaffold resorbs too fast disabling the mechanical support and affecting the growth of the new tissue [30]. Conversely, materials that degrade at a low rate can be highly porous to bring the degradation rate to an optimal level [30]. Additionally, higher specific surface area accelerates the degradation rate. The general consensus regarding the porosity needed for scaffold

application is that high porosity (over 80%) and bigger pores (>300 μm) favour new bone ingrowth, while mechanical strength dictates the limits of porosity and pore size [30, 37].

Scaffolds made of ceramics, polymers and combinations of these materials have been studied, and some have been used for clinical applications, such as blood vessels, drug and biomolecules delivery, cartilage, bone, and dental regeneration [5, 7, 8, 11-13, 17, 20, 21, 38-48]. Ceramic scaffolds are strong but brittle, while polymer scaffolds are mostly weak and ductile [5, 7, 8, 17, 20]. Ceramics, particularly bioglasses, in combination with polymers are being pursued with the aim of producing a composite biomaterial which will overcome the drawbacks of the individual materials, while resembling natural bone structure consisting of both inorganic and organic components [4, 5]. Yet, fulfilling all the requirements for an ideal scaffold, including high porosity, biocompatibility, biodegradability and suitable mechanical properties, while maintaining all the other parameters remains a challenge [17].

3. Bioglasses

Ceramics, and bioglasses among them, are of interest for tissue engineering applications on their own but also as a way to improve polymer properties (in particular bioactivity and mechanical strength) via the formation of composites which would suit biomedical applications. The first bioglass (45S5 Bioglass) was discovered by Larry Hench in 1969, and it was the first material which formed a good bond with bone [4]. 45S5 Bioglass has shown excellent biocompatibility and bioactivity *in vivo* as the layer of hydroxyapatite is formed on the surface [25]. 45S5 Bioglass also stimulates new bone formation with superior osteoinductive properties compared to hydroxyapatite and promotes angiogenesis (formation of blood vessels) [21, 49]. Dense 45S5 Bioglass is reported to have a compressive strength of 500 MPa, tensile strength 42 MPa, Young's modulus 35 GPa and fracture toughness of 0.7-1.1 MPa $m^{1/2}$ [26].

45S5 Bioglass was then followed by the development of other bioglass and bioglass-ceramic materials, such as 13-93 and apatite-wollastonite (A-W) glass-ceramic (Table 2) [4]. All bioglasses shown in Table 2 form a bond with bone, but the length of the bonding process, strength and thickness of the formed bond, as well as the mechanism of bond formation vary among different compositions, which in turn defines their application [50]. By modifying the composition, bioglasses can be designed to degrade at a controlled rate that matches the development of new bone tissue, without any toxic effects [25]. According to M. Pilia et al. [51] commonly used bioglasses have compressive strength 800-1200 MPa and Young's modulus 40-140 GPa but fracture toughness of around 2 MPa $m^{1/2}$. Furthermore, because many bioglasses have proven to have considerable antibacterial properties caused by cation leaching and a consequent rise in pH it makes them particularly useful in clinical

applications [4, 52-56]. For example, 45S5 Bioglass has been shown to kill *Enterococcus Faecalis* which is associated with failed root canal treatment while S53P4 has been shown to kill pathogens associated with enamel caries, root caries and periodontitis [4, 52-56].

Bioglass scaffolds have a number of excellent properties such as biocompatibility, bioactivity, degradability over time, and interconnected porosity suitable for bone ingrowth [4, 57, 58]. They can also be produced to have similar compressive strength to that of cancellous bone. For example, scaffolds made from 13-93 bioglass have been reported to have a compressive strength of 11 MPa, and an elastic modulus of 3 GPa (85% porosity with pore size of 100–500 μm) [26]. Generally, the literature shows that the compressive strength for bioglass scaffolds is in the range of 0.2-150 MPa (porosity 30-95%) and fracture toughness is 0.5-1 MPa $m^{1/2}$ which is low for load-bearing applications [59]. However, these scaffolds are not appropriate for applications that require flexibility or fatigue resistance as they are brittle [4]. Nevertheless, since bioglass is a material with high mechanical strength, it has its uses in combination with other materials where it serves as reinforcement, improving the properties of polymers and particularly to capitalise on the properties of the natural polymer chitosan [25]. Additionally, according to Jones [4] as well as Tajbakhsh and Hajiali [60], bioglasses can slow down the degradation of some polymers by releasing alkali metal ions that reduce the acidic pH produced by polymer degradation thereby acting as a buffer. The degradation rate is dependent on the percentage of bioglass in the composite [4, 60]. It has been suggested that the strong, covalent bond between the polymer and bioglass produces simultaneous degradation of the composite (section 5.1). However, it should be mentioned that other studies show that bioglass also increases swelling and degradation by making the composite more hydrophilic [4].

Table 2. Examples of bioglass compositions [50, 61, 62]

Name	45S5 Bioglass® (NovaBone)	45S5.4F Bioglass®	45B15S5 Bioglass®	52S4.6 Bioglass®	55S4.3 Bioglass®	KGC (Ceravital®)	KGS (Ceravital®)	KGy213 (Ceravital®)	S53P4 (AdminDent1®) (BonAlive®)	A-W Glass-ceramic (Cerabone®)	Ilmaplant-L_1® (Biovision GmbH)	Bioverit® (Vitron Spezialwerkstoffe GmbH)	13-93 glass®
SiO_2	45	45	30	52	55	46.2	46	38	53	34	44.3	19-54	54.6
P_2O_5	6	6	6	6	6	/	/	/	4	16.2	11.2	2-10	1.7
CaO	24.5	14.7	24.5	21	19.5	20.2	33	31	20	44.7	31.9	10-34	22.1
$Ca(PO_3)_2$	/	/	/	/	/	25.5	16	13.5	/	/	/	/	/
CAF_2	/	9.8	/	/	/	/	/	/	/	0.5	5	3-23	/
MgO	/	/	/	/	/	2.9	/	/	/	4.6	2.8	2-21	7.7
MgF_2	/	/	/	/	/	/	/	/	/	/	/	/	/
Na_2O	24.5	24.5	24.5	21	19.5	4.8	5	4	23	/	4.6	3-8	6
K_2O	/	/	/	/	/	0.4	/	/	/	/	0.2	/	7.9
Al_2O_3	/	/	/	/	/	/	/	7	/	/	/	8-15	/
B_2O_3	/	/	15	/	/	/	/	/	/	/	/	/	/
Ta_2O_5/ TiO_2	/	/	/	/	/	/	/	6.5	/	/	/	/	/
Type	Glass	Glass	Glass	Glass	Glass	Glass-ceramic	Glass-ceramic	Glass-ceramic	Glass	Glass-ceramic	Glass-ceramic	Glass-ceramic	Glass
Application	Middle-ear reconstruction, jaw defects filler	Maxillofacial reconstruction	Middle-ear reconstruction, dental implants	Percutaneous access devices, junction of spinal vertebrae	Dentistry	Middle-ear reconstruction	Middle-ear reconstruction	Middle-ear reconstruction, dental implants	Bony voids and gaps; bone cavity filling, cranio-maxillofacial	Vertebral prosthesis devices	Maxillofacial implants	Orthopaedic surgery (spacers), head and neck surgery (middle-ear implants), stomatology (tooth root and veneer	Approved for in vivo use (e.g. maxillary alveolar defects)

* Composition is given as wt%

4. Polymer/bioglass scaffolds

4.1 Types of polymer/bioglass scaffolds

In the quest to capitalise on the advantageous properties of polymers and bioglasses, there have been a number of successfully designed polymer/bioglass scaffolds. Based on their physical structure (appearance), the composite polymer/bioglass scaffolds reported so far can be classified in the following groups:

1) **Foam/sponge-like structures (scaffolds)** (Figure 1). A variety of methods have been used to develop these scaffolds including freeze-drying, foam replica, phase separation, particle leaching, dip- and slurry-coating and rapid prototyping [63-67]. Also, a large number of different polymers, both synthetic and natural, as well as different compositions of bioglasses have been used for this type of scaffold in order to obtain different designs [4, 16, 65, 68-78]. In general, scaffolds can be composed in two ways: a) they can consist of a matrix and a coating, where polymer foam can be coated with a bioglass slurry or a bioglass scaffold can be coated with polymer solution; or b) they can be formed as a monolith structure from bioglass particles dispersed in polymer solution [25, 64, 66, 67, 79].

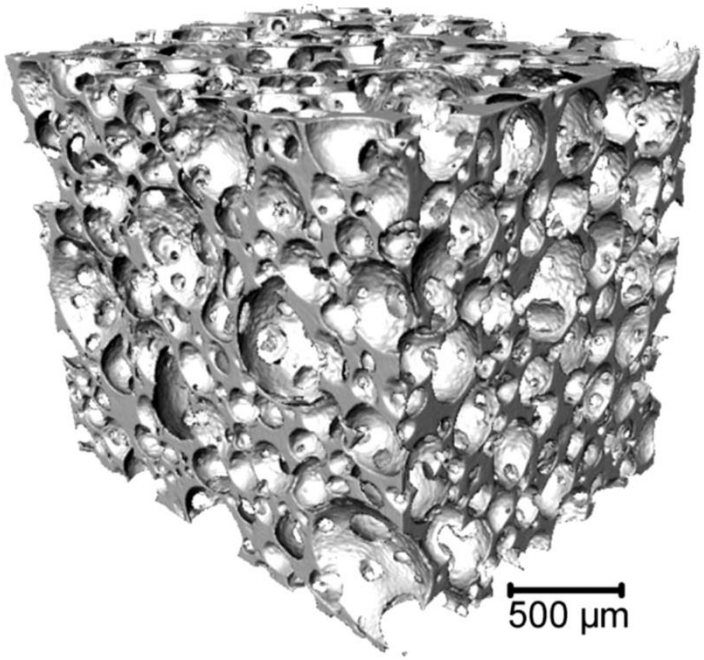

Figure 1: Example of a foam-like composite material reproduced with permission from [80].

2) **Fibre composites** (Figure 2). These are most widely synthesised via an electrospinning method, where materials can be mixed together prior to electrospinning or polymer fibres can be immersed in a bioglass slurry to coat the fibres [81-86]. A high surface area and high porosity make fibre scaffolds attractive for bone tissue applications, although reported mechanical properties are usually low or not stated [82, 84, 87]. Polyhydroxybutyrate (PHB)/poly(ε-caprolactone) (PCL)/58S bioglass (60 wt% SiO_2, 36 wt% CaO and 4 wt% P_2O_5) fibre scaffolds designed by Ding et al. exhibited a tensile strength of approximately 2 MPa and Young's modulus in the region of 67-87 MPa [88]. In another study by Foroughi et al, PHB/chitosan/45S5 Bioglass scaffolds showed a tensile strength in the region of 3 MPa and Young's modulus of 0.2 GPa (see Table 1 for comparison) [89].

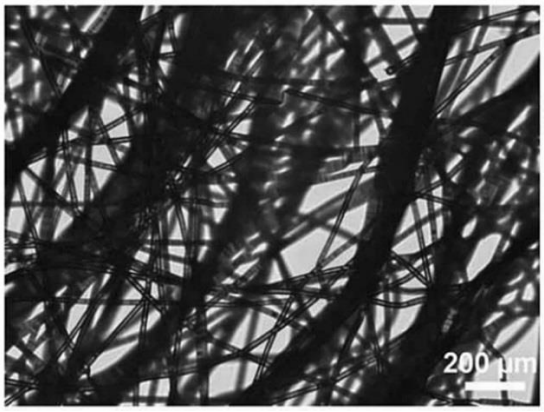

Figure 2. Fibre scaffold adapted with permission from [84]. Copyright 2013. Wiley Periodicals, Inc.

3) **Microsphere scaffolds**. Microspheres are mainly composed of polymers, but bioglass or other ceramics can be added to enhance mechanical strength and bioactivity of the materials, and control the degradation of the polymer. Microspheres can be fused together to form a 3D scaffold (Figure 3) [90-93]. This can be achieved by heating, using a solvent (e.g. methylene chloride and acetone), which is a milder method compared to heating, or via particle agglomeration techniques. This type of composite scaffold has been used for gene therapy and drug delivery in the antibiotic treatment of infected bone. Prior to fusing, the spheres are loaded with drugs which are released gradually over time. While microspheres offer the ability to encapsulate and release biomolecules and drugs, scaffolds composed of fused microspheres also possess porosity and mechanical support for loading cells, a useful feature for bone regeneration applications. This method of producing polymer/bioglass scaffolds is

reported to give improved mechanical properties similar to cancellous bone (compressive strength 4-8 MPa and compressive modulus 0.1-0.3 GPa) (see Table 1 for comparison) [94].

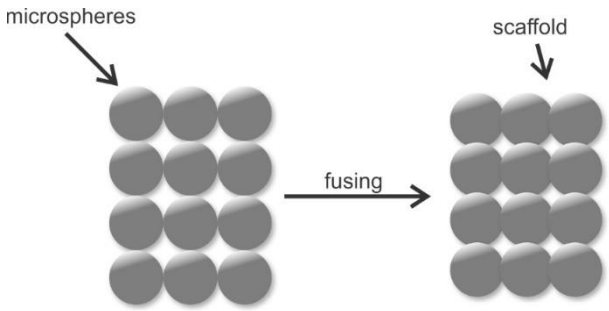

Figure 3. Microsphere scaffolds adapted with permission from [91]. Copyright 2014. The Royal Society of Chemistry

4) **Bilayer or multilayer scaffolds.** These scaffolds are designed for osteochondral applications which include both bone and cartilage tissue [11, 95, 96]. They consist of two or more layers; a bone-like layer (calcium phosphate material, polymer coated bioglass scaffold, or bioglass/polymer scaffold) and a cartilage layer (polymer material). The layered structure of the materials follows the natural architecture of a human osteochondral unit, where the bone-like part is used to support bone formation, while the cartilage-like part is used for guiding the formation of cartilage. Some scaffolds have an intermediate layer which serves as a 'glue' between the bioglass and polymer layers, and is usually made from a polymer or a polymer/hydroxyapatite composite (Figure 4) [11, 95]. A promising example was developed by Boccaccini et al. in which polyamide short fibres were applied as an external cartilage-like layer, to imitate collagen fibres, on a gelatin coated 45S5 Bioglass scaffold (Figure 5) [97].

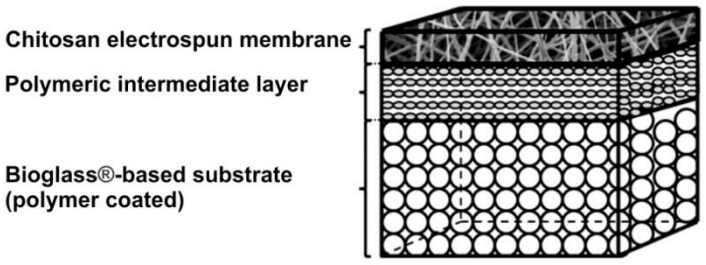

Figure 4. Multilayer composite scaffold made of polyvinyl alcohol (PVA) coated bioglass, polymeric (chitosan, alginate, gelatin or sucrose) and chitosan layers [95]. In this case, scaffolds were covered with polymeric solution by dipping and the chitosan membrane was fixed manually reproduced with permission from [95]. Copyright 2012. Elsevier B. V.

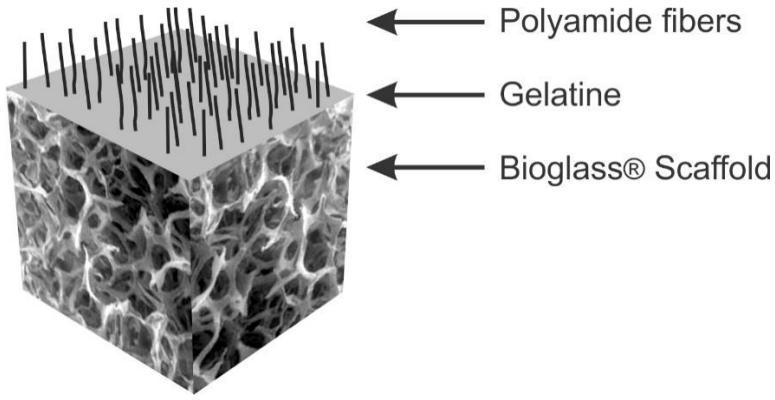

Figure 5: Bilayered gelatin coated 45S5 Bioglass scaffold with polyamide short fibres adapted with permission from [97]. Copyright 2015. Elsevier B. V.

5) **Cell-seeded scaffolds**. Here, cells are integrated into the composite scaffolds [48, 98, 99]. The presence of cells in engineered materials is found to improve osteogenesis *in vivo* so these scaffolds can serve as skeletons for cell incorporation and mechanical support [11, 100]. 3D printing or other additive manufacturing methods can be used for scaffold synthesis [98]. Figure 6 describes two different approaches for integrating cells into 3D printed materials. In the first approach cells are added to the hydrogel solutions (precursor materials) which are used to print 3D scaffolds. The second approach prints a 3D scaffold and then seeds it with cells [98]. The main issue with the first approach is the difficulty in designing scaffolds with the exact shape and mechanical properties that are required, whereas in the second approach, surface modification is necessary in order for the cells to be attached to the scaffold properly which complicates the fabrication process. Cells can also be seeded onto scaffolds fabricated via other routes, such as particulate leaching, thermally induced phase separation (TIPS), and the sponge replica method [48, 99, 101]. Microsphere scaffolds can also be loaded with cells and/or drugs. [93].

While multi-layered scaffolds are appropriate for osteochondral regeneration, polymer coated sponge-like bioglass scaffolds and microsphere scaffolds are suggested for use in bone defects as these types of structures give better mechanical properties than fibre scaffolds or polymer sponge scaffolds with dispersed bioglass. Additionally, cell-seeding can improve cell attachment to implanted scaffolds and enhance bone formation.

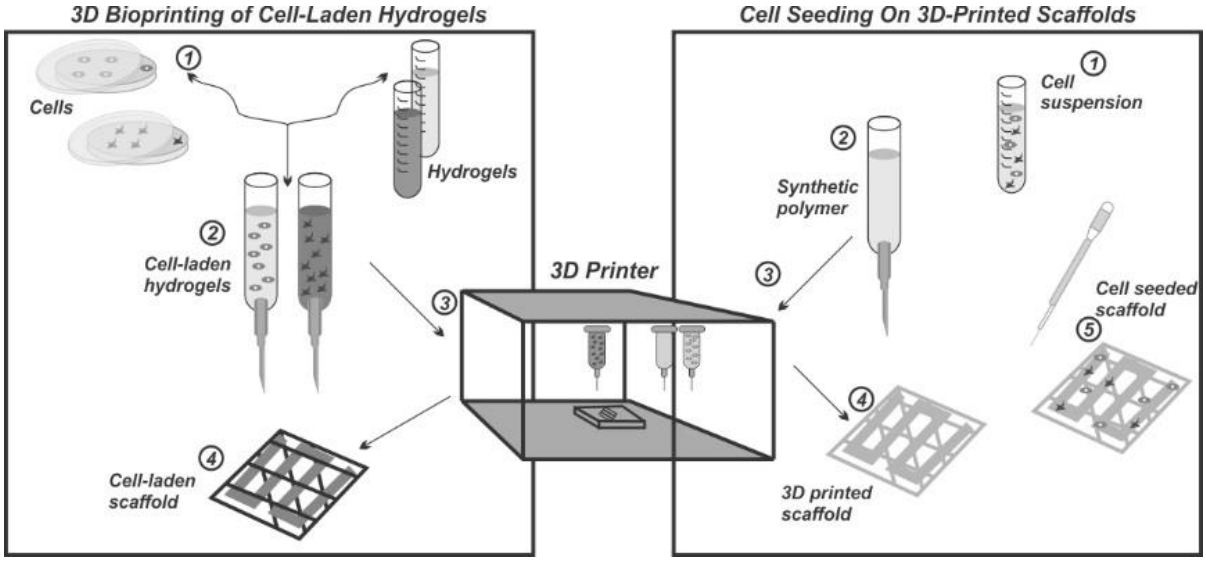

Figure 6: Cell-seeded scaffolds adapted with permission from [98]. Copyright 2015. Springer Science+Business Media New York

4.2 *In vivo* tested and commercially available polymer/bioglass composite scaffolds

A number of studies have reported *in vivo* testing of polymer/ceramic composite scaffolds designed for haemostasis (to stop bleeding), blood vessels, the delivery of drugs and biomolecules, and cartilage and bone regeneration applications [5, 11, 47, 48, 82, 86, 101-109]. Some of the materials for osteochondral regeneration with a multi-layered type of structure are under clinical trials. They consist of a combination of polymers (to mimic cartilage) and ceramics (to mimic bone tissue). ChondroMimetic™ is a composite based on *type I collagen*, chondroitin-6-sulfate, and calcium phosphate. TruFit™ consists of poly(lactic-co-glycolic acid) (PLGA) and poly(glycolic acid) (PGA) fibers, and calcium sulphate. MaioRegen® is made of three layers: *type I collagen*, a mixture of *type I collagen* and hydroxyapatite (60:40 wt% respectively) and a mixture of *type I collagen* and hydroxyapatite (30:70 wt% respectively) [11]. The use of multi-layered materials leads to optimal integration of repaired tissue with the native tissue in preclinical trials which examined several features including structural characteristics, mechanical properties, reconstruction of the bone, cellularity, the amount of defect filling area and the presence of defects [110]. The use of the natural polymer collagen (in ChondroMimetic™) was shown to have advantages over PLGA (in TruFit™). ChondroMimetic™ had better scores (according to Seller's scoring system for cartilage repair), rapid cell infiltration and bone matrix formation within the implanted materials, while TruFit™ had more bone defects in the form of cysts (bone cavities filled with fluid) [110]. The degradation of natural polymers is enzymatic with peptide and saccharide fragments being the products of degradation of collagen, which do not affect the local pH as they are naturally found in cartilage. On the other hand, degradation of PLGA leads to formation of acid oligomers (of lactic and glycolic acids) which have a detrimental effect on cells and

calcium phosphate formation leading to bone cysts. This was shown to induce a lack of integrity of the PLGA- materials with native tissue. Clinical reports of the multi-layered type of materials are contradictory and vary according to the study conducted [111-113]. Some studies report good follow-up and success in patients with patients being able to return to sporting activities, experiencing relief of symptoms and magnetic resonance imaging (MRI) showing complete filling of the defect and integration of the graft [112, 113]. For example, an Olympic fencer was able to compete 17 months after the implantation surgery [114]. However, the literature also reports a high rate of failure (formation of fibrous tissue and need for reoperation) when the same materials were used in other patients [111, 112]. The opposing outcomes are dependent on the age and previous activity levels of the patient as well as the different defect sites treated. As the data for the aforementioned multi-layered materials for cartilage repair does not give definitive answers on safety and efficiency in clinic, it suggests that novel materials (or improved existing materials) are needed.

Some commercially available composite scaffolds for bone tissue applications which have a sponge-type structure are shown in Table 3 [5, 12, 115]. Lerner *et al.* [116] reported that Vitoss (Orthovita), a scaffold of β-tricalcium phosphate and *type I collagen,* has similar results to autografts in scoliosis surgery. Radiographs showed complete and continuous fusion of the bone in both cases, while the 4/20 patients who received autographs suffered from donor site pain. One of the most successful commercially available bone graft products in the USA is Healos (DePuy Orthopaedics) which is a scaffold made of a matrix of cross-linked collagen fibres coated with hydroxyapatite [13]. Healos is successful because it requires less time for preparation but gives similar clinical results to autographs while avoiding donor site complications [117]. One big drawback of most of the commercially available polymer/bioglass composite scaffolds (see Table 3) is they use collagen, which can cause allergic reactions with some patients (although not common) and transmit pathogens because the collagen comes mainly from porcine and bovine sources [5, 118].

Table 3: Commercially available natural polymer/ceramic composite scaffolds [5, 12, 115]

Product	Composition and structure	Recommended use
Collagraft® (Zimmer)	Collagen I fibres and hydroxyapatite/tricalcium phosphate porous granules	Acute long bone fractures and traumatic osseous defects
Collapat® II (BioMet Inc.)	Collagen I and hydroxyapatite (hydroxyapatite granules dispersed in collagen structure)	Aseptic enclosed metaphyseal bone defects
FormaGraft® (Maxigen Biotech Inc.0	Collagen I and hydroxyapatite/β-tricalcium phosphate granules	Bone void filler
Integra Mozaik™ (Integra OrthoBiologics)	Collagen I and β-tricalcium phosphate	Bone void filler
CopiOs® (Zimmer)	Collagen I and calcium phosphate	Bone void filler
Biostite® (Vebas)	Collagen I/chondroitin-6-sulfate and hydroxyapatite	Filling of periodontal defects, pre-prosthetic osseous reconstruction, maxillo-facial reconstructive surgery
Bio-Oss Collagen® (Geistlich Biomaterials)	Porcine collagen and hydroxyapatite granules	Filling of periodontal defects, alveolar ridge
TricOs T® (Baxter)	Fibrin and hydroxyapatite/β-tricalcium phosphate granules	Bone void filler
RegenOss® (JRI Orthopaedics)	Collagen I fibres and Mg-enriched hydroxyapatite nano-crystals	Long bone fractures, revision hip arthroplasty to fill acetabular defects and spinal fusion
NanOss® Bioactive 3D (Pioneer surgical)	Collagen and nano hydroxyapatite granules	Bone void filler
Vitoss™ (Orthovita)	Collagen I and β-tricalcium phosphate	Spinal and trauma surgery
Healos® (DePuy Orthopaedics)	Matrix of cross-linked collagen fibres coated with hydroxyapatite	Spinal surgery

Even though products such as Healos and Vitoss have shown radiological and clinical results comparable to autografts, there are still some issues to be resolved such as the use of collagen, the need for further trials to determine clinical efficiency and insufficient mechanical properties for high load-bearing applications [5, 12, 117, 119]. The lack of high-load bearing applications of the developed polymer/ceramic scaffolds is because the bonding between the organic and inorganic phases in the current scaffolds is weak allowing the phases to degrade separately at different rates [4, 5]. This causes insufficient mechanical integrity resulting in inadequate mechanical properties [4]. Ultimately the scaffolds need better interfacial bonding between the polymer and bioglass phases to improve the mechanical properties.

5. Ongoing research aiming to overcome existing problems

5.1 Hybrids

It has been shown that microporosity is needed for good adhesion and linkage between the ceramic and polymer phases, which in turn improves mechanical performance [120]. Good dispersion of the bioglass particles in the polymer matrix is crucial to avoid aggregation and the consequent decrease in strength. Hybrid materials have the potential for a more homogeneous structure as well as the advantage over traditional composites because the constituents are interacting on a molecular level. A hybrid is defined as a composite of an inorganic and an organic material, where properties of the hybrid differ from the individual properties of the two materials because of the interactions between them. Hybrid materials are classed depending on the types of interactions occurring at the interface. *Class I hybrids* have weak bonds such as electrostatic, van der Waals and hydrogen bonds. This can be achieved by sol-gel synthesis [4, 121]. When covalent bonds between polymer and bioglass are present, the materials are called *Class II hybrids* [4, 122]. These materials have the potential for good bioactivity, improved mechanical properties and controlled and congruent degradation (the composition remains the same during degradation) [4].

Linkage can be achieved by using certain polymers with functional groups or by functionalization of the surface of either the bioglass or polymer before the sol-gel process [4, 123-130]. Silanes (glycidoxypropyltrimethoxysilane (GPTMS) and 3-aminopropyltriethoxysilane (APS)), hexamethylene diisocyanate (HMDI) and polyvinyl alcohol (PVA) are amongst the compounds used for developing *Class I* and *Class II* hybrid materials [123-130]. In a poly (L-lactide) (PLLA)/bioglass scaffold (with bioglass composition 4.6 wt% MgO, 44.7 wt% CaO, 34.0 wt% SiO_2, 16.2 wt% P_2O_5 and 0.5 wt% CaF_2), APS was used for functionalisation of the bioglass [127]. APS can bond to both bioglass and polylactide materials due to the presence of silanol groups which react with the bioglass, and amine groups which react with the carboxyl groups of hydrolysed PLLA to form hydrogen bonds. The use of APS to

functionalise the bioglass particles improved the bonding between the PLLA and bioglass particles which resulted in better incorporation of the bioglass particles into the PLLA matrix resulting in enhanced mechanical properties compared to non-functionalised samples [127]. In a different study, gelatin was functionalized with GPTMS and scaffolds with similar compression strength and modulus to cancellous bone were obtained [125]. Another interesting feature of these *Class II hybrid* scaffolds is that the apatite layer forms throughout the scaffold, whereas in purely bioglass scaffolds (bioglass containing 75 wt% SiO_2 and 25 wt% CaO) it was observed only on the surface [125].

Hexamethylene diisocyanate (HMDI) was used to functionalize PLLA to produce bonded PLLA/bioglass scaffolds (with bioglass composition: 58 wt% CaO, 29 wt% SiO_2, 13 wt% P_2O_5) [126]. Even though the Young's moduli of PLLA/bioglass scaffolds with or without HD were similar, higher tensile strength for the scaffold functionalized by diisocyanate was reported [126].

PVA/bioglass hybrid scaffolds (with bioglass composition: 58 wt% SiO_2, 33 wt% CaO, 9 wt% P_2O_5) were fabricated by the sol-gel method with the addition of a surfactant followed by ageing and drying [128, 130]. The hydroxyl group of PVA and the hydroxyl group of silanol groups, originating from the hydrolysis of the silicon precursor tetraethyl orthosilicate (TEOS), have been shown to react with each other to form bonds (Figure 7) [128]. These PVA/bioglass scaffolds were considered as potential candidates for bone tissue applications, however, so far, no exceptional properties in comparison to other known polymer/bioglass scaffolds have been reported [128, 130].

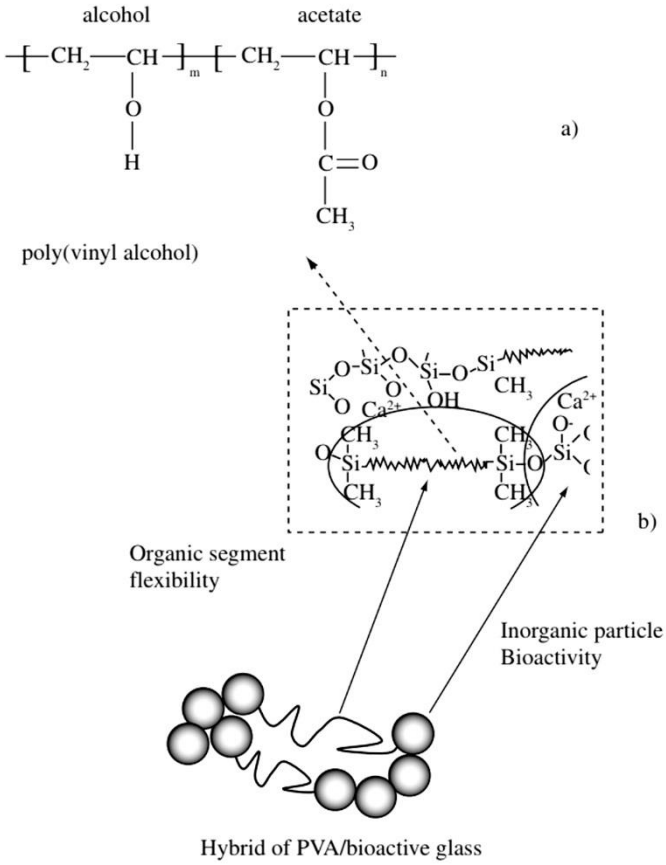

Figure 7: a) PVA with a variable hydrolysis degree and b) a hybrid structure reproduced from [128], available under a Creative Commons CC BY-NC (http://www.scielo.br/scielo.php?pid=S1516-14392007000100006&script=sci_abstract)

Lately, chitosan is drawing attention as a prospective component for *Class II hybrid* scaffolds with bioglasses. Glycidoxypropyltrimethoxysilane (GPTMS) was used for the functionalization of chitosan in order to form covalent bonds with bioglass via sol-gel synthesis [123]. Compared to chitosan and bioglass composites without GPTMS (*Class I hybrids*), specimens that were covalently bound (*Class II hybrids*) were less brittle. Figure 8 shows the functionalization of chitosan with GPTMS and formation of a chitosan/bioglass scaffold through the formation of a glass network thereby creating the hybrid. GPTMS is used as a cross-linking agent.

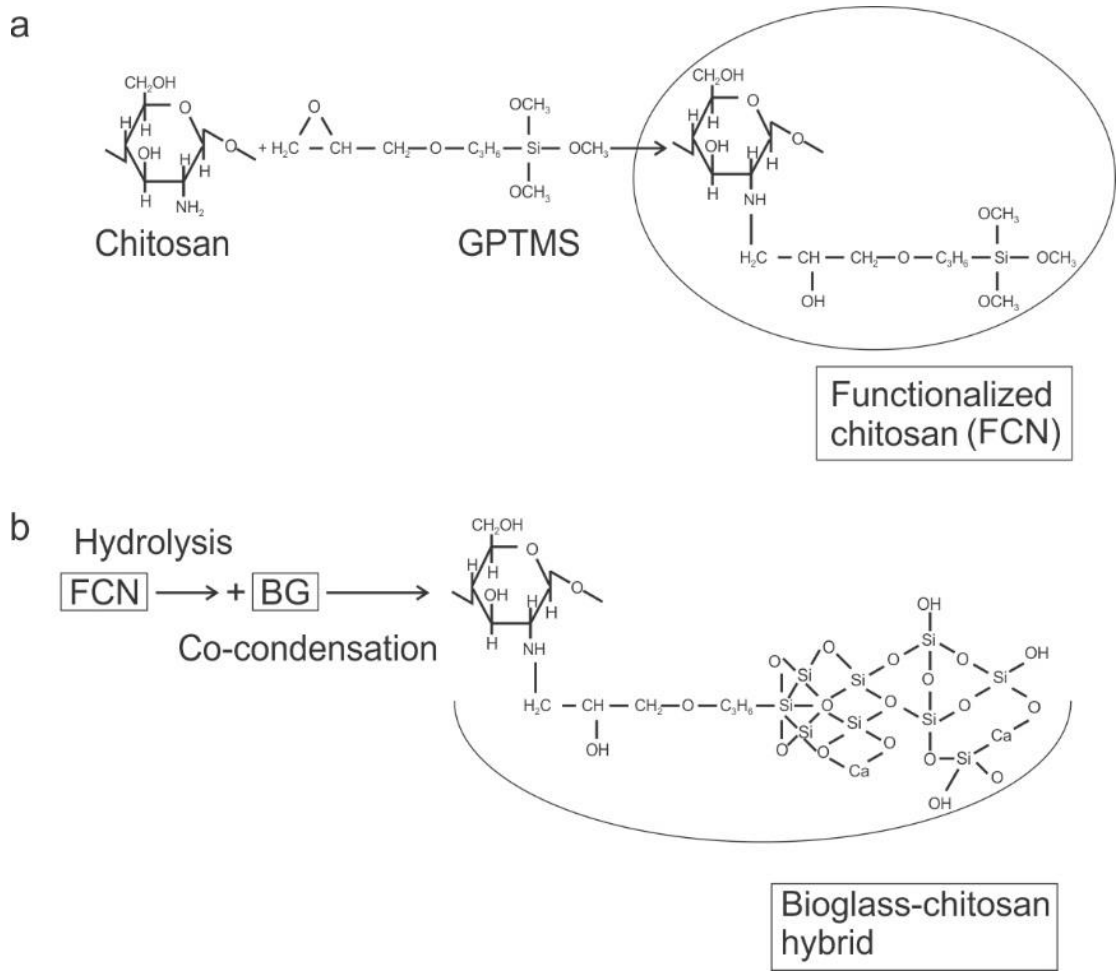

Figure 8: a) Functionalization of chitosan with GPTMS and b) formation of chitosan/bioglass hybrid scaffolds adapted with permission from [123]. Copyright 2015. Wiley Periodicals, Inc.

Another way to form a *Class II hybrid* involving chitosan is using GPTMS while bioglass is not added in this procedure (unlike in the method described in Figure 8). The cross-links form by reaction of the epoxy group of GPTMS with the amine group of chitosan. At the same time, the methoxysilane groups undergo hydrolysis to silanol groups which then condense to form a siloxane network [124]. These SiOH groups bring bioactivity to the polymer.

Further reports demonstrate PVA/chitosan/bioglass 60S (bioglass composition: 60.1 mol% SiO_2, 17.7 mol% Na_2O, 19.6 mol% CaO and 2.6% P_2O_5 mol%) hybrid scaffolds, fabricated with the addition of glutaraldehyde for cross-linking followed by freeze drying [129]. The untreated scaffolds showed toxicity, but after treatment with phosphate-buffered saline (PBS) cell viability increased.

In general, both *Class I* and *Class II hybrids* containing chitosan and bioglass are attractive for regenerative medicine applications, as they combine the good properties of both materials and potentially match the composition, structure and properties of human hard and soft tissue (cartilage

and bone). Some of the hybrids (APS or HMDI functionalised bioglass/PLLA, GPTMS functionalised chitosan/bioglass, and GPTMS functionalised gelatine/bioglass) showed improved mechanical properties in comparison to composites without functionalisation, but they are still below the high load-bearing region. While some designs of bioglass/chitosan composite scaffolds have been described in the literature, there is a need for further studies to realise their full potential. So far, chitosan matrices with bioglass as a coating or with bioglass nanoparticles within the polymer matrix have been reported [4, 16, 25, 39-44, 75, 77, 78, 123]. These have shown suitable biological and mechanical properties *in vitro* such as bioactivity, cell adhesion, no cell toxicity, adequate swelling and degradation and compressive strength in the region of cancellous bone, making them good candidates for further trials [16, 25, 75, 77]. Nanocomposite chitosan/bioglass scaffolds (with bioglass composition: 60 mol% SiO_2, 36 mol% CaO, 4 mol% P_2O_5) have also been reported [78]. While they had the appropriate pore size for bone growth, insufficient mechanical strength was reported [78]. Other studies tested chitosan/bioglass scaffolds for drug delivery *in vitro*, but mechanical properties were either not reported or low [39-44]. On the whole, even in the case of *Class II hybrid* chitosan/bioglass scaffolds, in which covalent bonding improved properties, the mechanical properties remained in the region of low load-bearing applications [123].

5.2 Potential of chitosan based materials

While drawing attention for use in hybrid scaffolds, chitosan is considered promising in the broader area of tissue engineering as it possesses a wide range of characteristics, such as non-toxicity, biocompatibility and biodegradability as well as having antibacterial properties while avoiding the problems linked to using collagen [25, 131]. Chitosan is a natural polycationic linear polysaccharide derived from chitin by deacetylation, although the process is never complete [131, 132]. Chitin is the second most abundant natural polysaccharide after cellulose and can be found in the exoskeletons of insects, shrimps, crabs, lobsters and in the cell walls of fungi [131]. As chitin is a waste material in the food industry, the production of chitosan is economically achievable and environmentally beneficial [132].

Chitosan has shown osteoconductive ability, as well as the ability to induce neovascularisation but shows very little osteoinductivity [131, 133]. One aspect to consider when using chitosan is its solubility, which is pH dependent (poor at neutral pH, and increasing as pH decreases) [133]. Chitosan on its own has insufficient mechanical strength and is easily degraded, especially in acidic surroundings, which can be an advantage for some *in vivo* applications but a drawback for others. It needs to be noted that chitosan can be unsuitable as blood-contacting material as it can cause thrombosis, aggregation of red blood cells and haemolysis [133].

Chitosan has been successfully employed in hydrogels which are 3D polymer networks that have an affinity for water absorption due to the presence of hydrophilic groups. When in an aqueous environment, water infiltrates the hydrogel network and causes swelling, while electrostatic or covalent bonds present in the network stop its dissolution [134-136]. While there are a number of cross-linkers used for chitosan hydrogel preparation that react with chitosan's amino groups (glutaraldehyde, formaldehyde, genipin, glyoxal, etc), these agents induce an increase in toxicity [134, 137]. On that front, genipin, a natural compound that is derived from Gardenia jasminoides fruits shows promise [137-139]. The advantage of genipin over the other commonly used cross-linkers is significantly lower toxicity (10 000 times less than glutaraldehyde) being nontoxic at concentrations below 0.5 μM, while the gels produced maintain a slower degradation rate, the same mechanical properties and, as an added bonus, have an anti-inflammatory effect [134, 137, 140, 141]. Genipin-cross-linked gels exhibit a 5 000 times higher cell proliferation rate and produce a reduced immune response in xenogenic matrices compared to gels cross-linked with glutaraldehyde [141, 142]. Currently, the significant drawback of using genipin is the high cost [139]. The reaction of genipin with chitosan, producing blue-coloured gels, is shown in Figure 9. It has been shown that variation in the quantity of genipin affects the structure and porosity of hydrogel networks [137, 143]. Genipin can self-polymerise, especially in a higher pH environment, which reduces the degree of cross-linking but introduces a further degree of freedom when it comes to pore geometry and size [139, 144]. By varying the reaction conditions, such as temperature or pH, the obtained genipin cross-linked chitosan can express different properties [145]. By increasing the temperature, denser and more cross-linked chitosan networks can be made [146]. Under variable pH conditions, different degrees of cross-linking can be obtained which can influence gel properties such as mechanical properties and the rate of drug release [147-149]. As mentioned previously, it is important to have the capacity to tailor porosity and structure of the material in order to gain desirable properties such as biocompatibility, controlled degradation with a controlled rate and suitable mechanical properties.

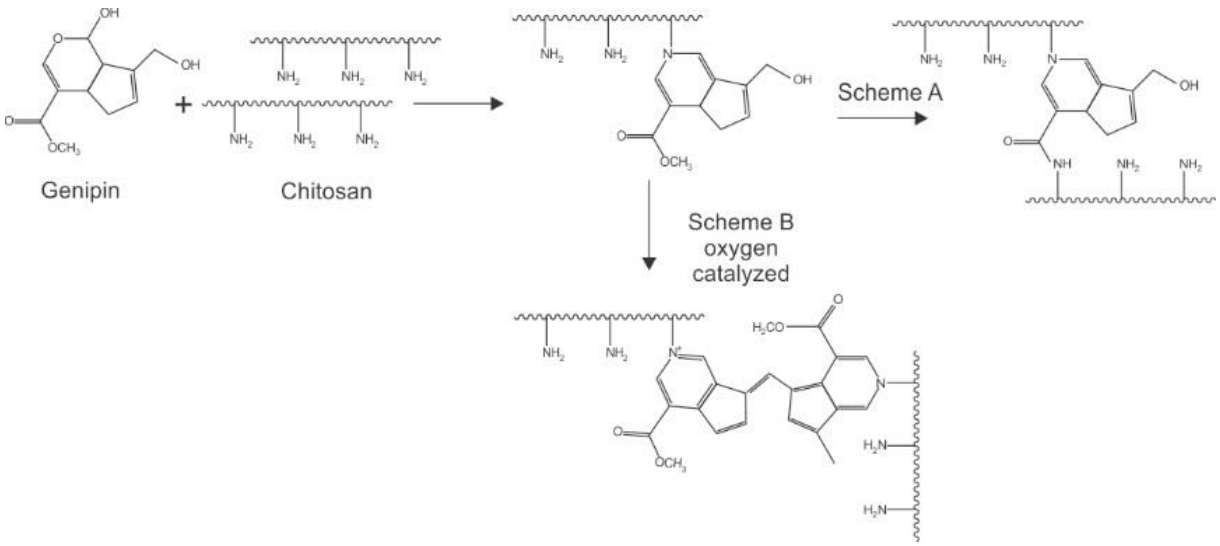

Figure 9: Possible genipin/chitosan cross-linking reactions adapted with permission from [150]. Copyright 2015. Wiley Periodicals, Inc.

5. Conclusion

Composite materials are pursued for bone tissue engineering as they resemble natural human bone consisting of organic and inorganic parts. The composite scaffolds reported so far are suitable for non-load-bearing or low load-bearing applications, for example for biomolecule delivery, as bone defect fillers and for maxillofacial procedures. Currently, the materials in use have mechanical properties below or at the lower limit of human cortical bone. The weak point of the composites is the weak interfacial bonding between the polymer and bioglass phases which jeopardises the mechanical properties of the composite.

One way to improve the bonding between the organic and inorganic phase of the composites is to introduce a covalent bond between the phases (formation of *Class II hybrids*). This results in a stronger interface, preferably enabling the two phases to degrade simultaneously. It is also important to choose the appropriate structures for developing scaffolds in order to achieve better mechanical properties, for example hydrogel coated ceramic scaffolds or microsphere scaffolds exhibit higher mechanical strength.

As an alternative to collagen, using chitosan for developing scaffolds with bioglass will avoid problems such as allergy and pathogen transmission. Chitosan has suitable specific properties such as biocompatibility, biodegradability, antibacterial properties and it is abundant in nature. Introducing cross-linking agents to the polymers improves mechanical properties of the polymer phase. So far, only a few studies have reported the use of cross-linking agents (usually glutaraldehyde) in

polymer/bioglass, and specifically chitosan/bioglass, scaffolds. Given that improved synthesis methods will decrease the cost of genipin manufacturing, this cross-linking agent could be a major player in improving chitosan-based composite scaffolds with low toxicity.

The literature suggests that combined methods need to be taken into account (new ways of forming covalent bond with novel coupling agents, cross-linking of polymer hydrogels, scaffold coating or multi-layered scaffolds) in order to develop appropriate scaffolds for high-load bearing implants. Replacement of collagen with other natural polymers, such as chitosan, should be considered. It should be emphasized that even when *in vivo* tests support the use of certain materials, clinical trials might show unexpected or inconclusive results and vary from patient to patient, which remains an issue.

6. Acknowledgements

This work was supported by UK Engineering and Physical Sciences Research Council (EPSRC) grant number EP/N033655/1, NUORS Award and CEAMIPS funding.

7. References

[1] M.N.V.R. Kumar, R.A.A. Muzzarelli, C. Muzzarelli, H. Sashiwa, A.J. Domb, Chitosan chemistry and pharmaceutical perspectives, Chem Rev, 104 (2004) 6017-6084, https://doi.org/10.1021/cr030441b.

[2] marketsandmarkets.com, *Biomaterials market by type of materials (metallic, ceramic, polymers, natural) & application (cardiovascular, orthopedic, dental, plastic surgery, wound healing, neurology, tissue engineering, ophthalmology) - global forecast to 2021*. 2016 [cited 2018 2 July]; Available from: http://www.marketsandmarkets.com/Market-Reports/biomaterials-393.html.

[3] S. Wu, X. Liu, K.W.K. Yeung, C. Liu, X. Yang, Biomimetic porous scaffolds for bone tissue engineering, Mater Sci Eng R Rep, 80 (2014) 1-36, http://dx.doi.org/10.1016/j.mser.2014.04.001.

[4] J.R. Jones, Review of bioactive glass: From Hench to hybrids, Acta Biomater, 9 (2013) 4457-4486, http://dx.doi.org/10.1016/j.actbio.2012.08.023.

[5] R. Yunus Basha, T.S. Sampath Kumar, M. Doble, Design of biocomposite materials for bone tissue regeneration, Mater Sci Eng C, 57 (2015) 452-463, http://dx.doi.org/10.1016/j.msec.2015.07.016.

[6] K.K. Mallick, J. Winnett, Preparation and characterization of porous Bioglass® and PLLA scaffolds for tissue engineering applications, J Am Ceram Soc, 95 (2012) 2680-2686, https://doi.org/10.1111/j.1551-2916.2012.05071.x.

[7] E. Garcia-Gareta, M.J. Coathup, G.W. Blunn, Osteoinduction of bone grafting materials for bone repair and regeneration, Bone, 81 (2015) 112-121, https://doi.org/10.1016/j.bone.2015.07.007.

[8] F. Asa'ad, G. Pagni, S.P. Pilipchuk, A.B. Gianni, W.V. Giannobile, G. Rasperini, 3D-printed scaffolds and biomaterials: Review of alveolar bone augmentation and periodontal regeneration applications, Int J Dent, 2016 (2016) 1-15, https://doi.org/10.1155/2016/1239842.
[9] P. Kumar, B. Vinitha, G. Fathima, Bone grafts in dentistry, J Pharm Bioallied Sci, 5 (2013) S125-S127, https://doi.org/10.4103/0975-7406.113312.
[10] T.A. van Vugt, J. Geurts, J.J. Arts, Clinical application of antimicrobial bone graft substitute in osteomyelitis treatment: A systematic review of different bone graft substitutes available in clinical treatment of osteomyelitis, Biomed Res Int, 2016 (2016) 1-9, https://doi.org/10.1155/2016/6984656.
[11] S. Lopa, H. Madry, Bioinspired scaffolds for osteochondral regeneration, Tissue Eng A, 20 (2014) 2052-2076, https://doi.org/10.1089/ten.tea.2013.0356.
[12] T. Kurien, R.G. Pearson, B.E. Scammell, Bone graft substitutes currently available in orthopaedic practice. The evidence for their use, Bone Joint J, 95-B (2013) 583-597, https://doi.org/10.1302/0301-620x.95b5.30286.
[13] R. Mishra, T. Bishop, I.L. Valerio, J.P. Fisher, D. Dean, The potential impact of bone tissue engineering in the clinic, Regen Med, 11 (2016) 571-587, https://doi.org/10.2217/rme-2016-0042.
[14] V. Guarino, T. Caputo, R. Altobelli, L. Ambrosio, Degradation properties and metabolic activity of alginate and chitosan polyelectrolytes for drug delivery and tissue engineering applications, AIMS Mater Sci, 2 (2015) 497-502, https://doi.org/10.3934/matersci.2015.4.497.
[15] I. Manavitehrani, A. Fathi, H. Badr, S. Daly, A. Negahi Shirazi, F. Dehghani, Biomedical applications of biodegradable polyesters, Polymers, 8 (2016) 20-51, https://doi.org/10.3390/polym8010020.
[16] M. Pourhaghgouy, A. Zamanian, M. Shahrezaee, M.P. Masouleh, Physicochemical properties and bioactivity of freeze-cast chitosan nanocomposite scaffolds reinforced with bioactive glass, Mater Sci Eng C, 58 (2016) 180-186, http://dx.doi.org/10.1016/j.msec.2015.07.065.
[17] S. Bose, M. Roy, A. Bandyopadhyay, Recent advances in bone tissue engineering scaffolds, Trends Biotechnol, 30 (2012) 546-554, http://dx.doi.org/10.1016/j.tibtech.2012.07.005.
[18] Y.-M. Lee, Y.-J. Park, S.-J. Lee, Y. Ku, S.-B. Han, P.R. Klokkevold, S.-M. Choi, C.-P. Chung, Tissue engineered bone formation using chitosan/tricalcium phosphate sponges, J Periodontol, 71 (2000) 410-417, https://doi.org/10.1902/jop.2000.71.3.410.
[19] M.A. Rebelo, T.F.R. Alves, R. de Lima, J.M. Oliveira, Jr., M.M.D.C. Vila, V.M. Balcão, P. Severino, M.V. Chaud, Scaffolds and tissue regeneration: An overview of the functional properties of selected organic tissues, J Biomed Mater Res B Appl Biomater, 104 (2016) 1483-1494, https://doi.org/10.1002/jbm.b.33482.

[20] K.S. Griffin, K.M. Davis, T.O. McKinley, J.O. Anglen, T.-M.G. Chu, J.D. Boerckel, M.A. Kacena, Evolution of bone grafting: Bone grafts and tissue engineering strategies for vascularized bone regeneration, Clin Rev Bone Miner Metab, 13 (2015) 232-244, https://doi.org/10.1007/s12018-015-9194-9.

[21] S. Deepthi, J. Venkatesan, S.-K. Kim, J.D. Bumgardner, R. Jayakumar, An overview of chitin or chitosan/nano ceramic composite scaffolds for bone tissue engineering, Int J Biol Macromol, 93 (2016) 1338-1353, http://dx.doi.org/10.1016/j.ijbiomac.2016.03.041.

[22] T. Albrektsson, C. Johansson, Osteoinduction, osteoconduction and osseointegration, European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society, 10 (2001) S96-S101, https://doi.org/10.1007/s005860100282.

[23] T. Kokubo, H. Takadama, How useful is SBF in predicting in vivo bone bioactivity?, Biomaterials, 27 (2006) 2907-2915, https://doi.org/10.1016/j.biomaterials.2006.01.017.

[24] I.D. Thompson, L.L. Hench, Mechanical properties of bioactive glasses, glass-ceramics and composites, Proc Inst Mech Eng H, 212 (1998) 127-136, https://doi.org/10.1243/0954411981533908.

[25] J. Yang, T. Long, N.-F. He, Y.-P. Guo, Z.-A. Zhu, Q.-F. Ke, Fabrication of a chitosan/bioglass three-dimensional porous scaffold for bone tissue engineering applications, J Mater Chem B, 2 (2014) 6611-6618, https://doi.org/10.1039/C4TB00940A.

[26] L.C. Gerhardt, A.R. Boccaccini, Bioactive Glass and Glass-Ceramic Scaffolds for Bone Tissue Engineering, Materials (Basel), 3 (2010) 3867-3910, https://doi.org/10.3390/ma3073867.

[27] A. Kraitzer, *Bone grafts engineering*. 2012 [cited 2017 25 May]; Available from: https://www.slideshare.net/amirkraitzer/bone-grafts-enineering.

[28] C.M. Murphy, M.G. Haugh, F.J. O'Brien, The effect of mean pore size on cell attachment, proliferation and migration in collagen–glycosaminoglycan scaffolds for bone tissue engineering, Biomaterials, 31 (2010) 461-466, http://dx.doi.org/10.1016/j.biomaterials.2009.09.063.

[29] J.R. Woodard, A.J. Hilldore, S.K. Lan, C.J. Park, A.W. Morgan, J.A.C. Eurell, S.G. Clark, M.B. Wheeler, R.D. Jamison, A.J. Wagoner Johnson, The mechanical properties and osteoconductivity of hydroxyapatite bone scaffolds with multi-scale porosity, Biomaterials, 28 (2007) 45-54, http://dx.doi.org/10.1016/j.biomaterials.2006.08.021.

[30] V. Karageorgiou, D. Kaplan, Porosity of 3D biomaterial scaffolds and osteogenesis, Biomaterials, 26 (2005) 5474-5491, http://dx.doi.org/10.1016/j.biomaterials.2005.02.002.

[31] S. Lee, M. Porter, S. Wasko, G. Lau, P.-Y. Chen, E.E. Novitskaya, A.P. Tomsia, A. Almutairi, M.A. Meyers, J. McKittrick, Potential bone replacement materials prepared by two methods, MRS Proceedings, 1418 (2012) mrsf11-1418-mm1406-1402, https://doi.org/10.1557/opl.2012.671.
[32] K. Rezwan, Q.Z. Chen, J.J. Blaker, A.R. Boccaccini, Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering, Biomaterials, 27 (2006) 3413-3431, https://doi.org/10.1016/j.biomaterials.2006.01.039.
[33] H. Yuan, K. Kurashina, J.D. de Bruijn, Y. Li, K. de Groot, X. Zhang, A preliminary study on osteoinduction of two kinds of calcium phosphate ceramics, Biomaterials, 20 (1999) 1799-1806, http://dx.doi.org/10.1016/S0142-9612(99)00075-7.
[34] P. Habibovic, H. Yuan, C.M. van der Valk, G. Meijer, C.A. van Blitterswijk, K. de Groot, 3D microenvironment as essential element for osteoinduction by biomaterials, Biomaterials, 26 (2005) 3565-3575, https://doi.org/10.1016/j.biomaterials.2004.09.056.
[35] B. Thavornyutikarn, P. Tesavibul, K. Sitthiseripratip, N. Chatarapanich, B. Feltis, P.F.A. Wright, T.W. Turney, Porous 45S5 Bioglass(R)-based scaffolds using stereolithography: Effect of partial pre-sintering on structural and mechanical properties of scaffolds, Mater Sci Eng C Mater Biol Appl, 75 (2017) 1281-1288, https://doi.org/10.1016/j.msec.2017.03.001.
[36] J.L. Simon, T.D. Roy, J.R. Parsons, E.D. Rekow, V.P. Thompson, J. Kemnitzer, J.L. Ricci, Engineered cellular response to scaffold architecture in a rabbit trephine defect, J Biomed Mater Res A, 66A (2003) 275-282, https://doi.org/10.1002/jbm.a.10569.
[37] I. Ochoa, J.A. Sanz-Herrera, J.M. García-Aznar, M. Doblaré, D.M. Yunos, A.R. Boccaccini, Permeability evaluation of 45S5 Bioglass®-based scaffolds for bone tissue engineering, J Biomech, 42 (2009) 257-260, http://dx.doi.org/10.1016/j.jbiomech.2008.10.030.
[38] J. Hum, A.R. Boccaccini, Bioactive glasses as carriers for bioactive molecules and therapeutic drugs: A review, J Mater Sci Mater Med, 23 (2012) 2317-2333, https://doi.org/10.1007/s10856-012-4580-z.
[39] C. Soundrapandian, A. Mahato, B. Kundu, S. Datta, B. Sa, D. Basu, Development and effect of different bioactive silicate glass scaffolds: In vitro evaluation for use as a bone drug delivery system, J Mech Behav Biomed Mater, 40 (2014) 1-12, https://doi.org/10.1016/j.jmbbm.2014.08.007.
[40] E. Wers, H. Oudadesse, B. Lefeuvre, O. Merdrignac-Conanec, A. Barroug, Evaluation of the kinetic and relaxation time of gentamicin sulfate released from hybrid biomaterial bioglass-chitosan scaffolds, Appl Surf Sci, 353 (2015) 200-208, https://doi.org/10.1016/j.apsusc.2015.06.146.
[41] K. Nazemi, P. Azadpour, F. Moztarzadeh, A.M. Urbanska, M. Mozafari, Tissue-engineered chitosan/bioactive glass bone scaffolds integrated with PLGA nanoparticles: A therapeutic design for

on-demand drug delivery, Mater Lett, 138 (2015) 16-20, https://doi.org/10.1016/j.matlet.2014.09.086.
[42] A.A. Mostafa, M.M.H. El-Sayed, A.A. Mahmoud, A.M. Gamal-Eldeen, Bioactive/natural polymeric scaffolds loaded with ciprofloxacin for treatment of osteomyelitis, AAPS PharmSciTech, 18 (2016) 1056-1069, https://doi.org/10.1208/s12249-016-0605-0.
[43] M.I. Abdelfattah, S.A. Nasry, A.A. Mostafa, Characterization and cytotoxicity analysis of a ciprofloxacin loaded chitosan/bioglass scaffold on cultured human periodontal ligament stem cells: A preliminary report, Open Access Maced J Med Sci, 4 (2016) 461-467, https://doi.org/10.3889/oamjms.2016.052.
[44] C. Soundrapandian, S. Datta, B. Kundu, D. Basu, B. Sa, Porous bioactive glass scaffolds for local drug delivery in osteomyelitis: Development and in vitro characterization, AAPS PharmSciTech, 11 (2010) 1675-1683, https://doi.org/10.1208/s12249-010-9550-5.
[45] A. Shamosi, D. Mehrabani, M. Azami, S. Ebrahimi-Barough, V. Siavashi, H. Ghanbari, E. Sharifi, R. Roozafzoon, J. Ai, Differentiation of human endometrial stem cells into endothelial-like cells on gelatin/chitosan/bioglass nanofibrous scaffolds, Artif Cells Nanomed Biotechnol, 45 (2017) 163-173, https://doi.org/10.3109/21691401.2016.1138493.
[46] W.J. Habraken, J.G. Wolke, J.A. Jansen, Ceramic composites as matrices and scaffolds for drug delivery in tissue engineering, Adv Drug Deliv Rev, 59 (2007) 234-248, https://doi.org/10.1016/j.addr.2007.03.011.
[47] J.K. Leach, D. Kaigler, Z. Wang, P.H. Krebsbach, D.J. Mooney, Coating of VEGF-releasing scaffolds with bioactive glass for angiogenesis and bone regeneration, Biomaterials, 27 (2006) 3249-3255, https://doi.org/10.1016/j.biomaterials.2006.01.033.
[48] J. Wu, K. Xue, H. Li, J. Sun, K. Liu, Improvement of PHBV scaffolds with bioglass for cartilage tissue engineering, PLoS ONE, 8 (2013) e71563, https://doi.org/10.1371/journal.pone.0071563.
[49] F. Baino, S. Fiorilli, C. Vitale-Brovarone, Bioactive glass-based materials with hierarchical porosity for medical applications: Review of recent advances, Acta Biomater, 42 (2016) 18-32, http://dx.doi.org/10.1016/j.actbio.2016.06.033.
[50] S. Hampshire, *Bioglasses and bioglass-ceramics*. 2015 [cited 2017 25 October]; Available from: http://www.cost-newgen.org/wp-content/uploads/2016/06/3B-Bioglasses-and-glass-ceramics-S-Hampshire.pdf.
[51] M. Pilia, T. Guda, M. Appleford, Development of composite scaffolds for load-bearing segmental bone defects, Biomed Res Int, 2013 (2013) 1-15, https://doi.org/10.1155/2013/458253.

[52] I. Allan, H. Newman, M. Wilson, Antibacterial activity of particulate Bioglass® against supra- and subgingival bacteria, Biomaterials, 22 (2001) 1683-1687, http://dx.doi.org/10.1016/S0142-9612(00)00330-6.
[53] O. Leppäranta, M. Vaahtio, T. Peltola, D. Zhang, L. Hupa, M. Hupa, H. Ylänen, J.I. Salonen, M.K. Viljanen, E. Eerola, Antibacterial effect of bioactive glasses on clinically important anaerobic bacteria in vitro, J Mater Sci Mater Med, 19 (2008) 547-551, https://doi.org/10.1007/s10856-007-3018-5.
[54] D. Zhang, O. Leppäranta, E. Munukka, H. Ylänen, M.K. Viljanen, E. Eerola, M. Hupa, L. Hupa, Antibacterial effects and dissolution behavior of six bioactive glasses, J Biomed Mater Res A, 93A (2010) 475-483, https://doi.org/10.1002/jbm.a.32564.
[55] E. Munukka, O. Leppäranta, M. Korkeamäki, M. Vaahtio, T. Peltola, D. Zhang, L. Hupa, H. Ylänen, J.I. Salonen, M.K. Viljanen, E. Eerola, Bactericidal effects of bioactive glasses on clinically important aerobic bacteria, J Mater Sci Mater Med, 19 (2008) 27-32, https://doi.org/10.1007/s10856-007-3143-1.
[56] S. Hu, J. Chang, M. Liu, C. Ning, Study on antibacterial effect of 45S5 Bioglass®, J Mater Sci Mater Med, 20 (2009) 281-286, https://doi.org/10.1007/s10856-008-3564-5.
[57] G. Poologasundarampillai, P.D. Lee, C. Lam, A.-M. Kourkouta, J.R. Jones, Compressive strength of bioactive sol–gel glass foam scaffolds, Int J Appl Glass Sci, 7 (2016) 229-237, https://doi.org/10.1111/ijag.12211.
[58] E. Boccardi, V. Melli, G. Catignoli, L. Altomare, M.T. Jahromi, M. Cerruti, L.-P. Lefebvre, L.D. Nardo, Study of the mechanical stability and bioactivity of Bioglass ® based glass-ceramic scaffolds produced via powder metallurgy-inspired technology, Biomed Mater, 11 (2016) 015005, http://stacks.iop.org/1748-605X/11/i=1/a=015005.
[59] Q. Fu, E. Saiz, M.N. Rahaman, A.P. Tomsia, Toward Strong and Tough Glass and Ceramic Scaffolds for Bone Repair, Adv Funct Mater, 23 (2013) 5461-5476, https://doi.org/10.1002/adfm.201301121.
[60] S. Tajbakhsh, F. Hajiali, A comprehensive study on the fabrication and properties of biocomposites of poly(lactic acid)/ceramics for bone tissue engineering, Mater Sci Eng C, 70 (2017) 897-912, http://dx.doi.org/10.1016/j.msec.2016.09.008.
[61] L.L. Hench, Bioactive glasses and glass-ceramics, Mater Sci Forum, 293 (1999) 37-64, https://doi.org/10.4028/www.scientific.net/MSF.293.37.
[62] S. Nandi, B. Kundu, S. Datta, *Development and applications of varieties of bioactive glass compositions in dental surgery, third generation tissue engineering, orthopaedic surgery and as drug delivery system*. 2011 [cited 2018 10 July]; Available from:

https://www.intechopen.com/books/biomaterials-applications-for-nanomedicine/development-and-applications-of-varieties-of-bioactive-glass-compositions-in-dental-surgery-third-ge.
[63] Q.Z. Chen, A.R. Boccaccini, Poly(D,L-lactic acid) coated 45S5 Bioglass-based scaffolds: Processing and characterization, J Biomed Mater Res A, 77 (2006) 445-457, https://doi.org/10.1002/jbm.a.30636.
[64] D. Durgalakshmi, S. Balakumar, Analysis of solvent induced porous PMMA-bioglass monoliths by the phase separation method-mechanical and in vitro biocompatible studies, Phys Chem Chem Phys, 17 (2015) 1247-1256, https://doi.org/10.1039/c4cp03515a.
[65] E. Zeimaran, S. Pourshahrestani, I. Djordjevic, B. Pingguan-Murphy, N.A. Kadri, M.R. Towler, Bioactive glass reinforced elastomer composites for skeletal regeneration: A review, Mater Sci Eng C Mater Biol Appl, 53 (2015) 175-188, https://doi.org/10.1016/j.msec.2015.04.035.
[66] A. Motealleh, S. Eqtesadi, F.H. Perera, A. Pajares, F. Guiberteau, P. Miranda, Understanding the role of dip-coating process parameters in the mechanical performance of polymer-coated bioglass robocast scaffolds, J Mech Behav Biomed Mater, 64 (2016) 253-261, https://doi.org/10.1016/j.jmbbm.2016.08.004.
[67] M. Dziadek, J. Pawlik, E. Menaszek, E. Stodolak-Zych, K. Cholewa-Kowalska, Effect of the preparation methods on architecture, crystallinity, hydrolytic degradation, bioactivity, and biocompatibility of PCL/bioglass composite scaffolds, J Biomed Mater Res B, 103 (2015) 1580-1593, https://doi.org/10.1002/jbm.b.33350.
[68] A. Larranaga, E. Diamanti, E. Rubio, T. Palomares, A. Alonso-Varona, P. Aldazabal, F.J. Martin, J.R. Sarasua, A study of the mechanical properties and cytocompatibility of lactide and caprolactone based scaffolds filled with inorganic bioactive particles, Mater Sci Eng C Mater Biol Appl, 42 (2014) 451-460, https://doi.org/10.1016/j.msec.2014.05.061.
[69] V. Maquet, A.R. Boccaccini, L. Pravata, I. Notingher, R. Jerome, Porous poly(alpha-hydroxyacid)/bioglass composite scaffolds for bone tissue engineering. I: Preparation and in vitro characterisation, Biomaterials, 25 (2004) 4185-4194, https://doi.org/10.1016/j.biomaterials.2003.10.082.
[70] O. Bretcanu, A.R. Boccaccini, V. Salih, Poly-dl-lactic acid coated Bioglass® scaffolds: Toughening effects and osteosarcoma cell proliferation, J Mater Sci, 47 (2012) 5661-5672, https://doi.org/10.1007/s10853-012-6315-4.
[71] J. Venkatesan, I. Bhatnagar, P. Manivasagan, K.H. Kang, S.K. Kim, Alginate composites for bone tissue engineering: A review, Int J Biol Macromol, 72 (2015) 269-281, https://doi.org/10.1016/j.ijbiomac.2014.07.008.

[72] Q. Yao, W. Li, S. Yu, L. Ma, D. Jin, A.R. Boccaccini, Y. Liu, Multifunctional chitosan/polyvinyl pyrrolidone/45S5 Bioglass® scaffolds for MC3T3-E1 cell stimulation and drug release, Mater Sci Eng C Mater Biol Appl, 56 (2015) 473-480, https://doi.org/10.1016/j.msec.2015.06.046.
[73] C.D. Moreira, S.M. Carvalho, H.S. Mansur, M.M. Pereira, Thermogelling chitosan-collagen-bioactive glass nanoparticle hybrids as potential injectable systems for tissue engineering, Mater Sci Eng C Mater Biol Appl, 58 (2016) 1207-1216, https://doi.org/10.1016/j.msec.2015.09.075.
[74] P. Gentile, M. Mattioli-Belmonte, V. Chiono, C. Ferretti, F. Baino, C. Tonda-Turo, C. Vitale-Brovarone, I. Pashkuleva, R.L. Reis, G. Ciardelli, Bioactive glass/polymer composite scaffolds mimicking bone tissue, J Biomed Mater Res A, 100 (2012) 2654-2667, https://doi.org/10.1002/jbm.a.34205.
[75] C.O. Correia, Á.J. Leite, J.F. Mano, Chitosan/bioactive glass nanoparticles scaffolds with shape memory properties, Carbohydr Polym, 123 (2015) 39-45, http://dx.doi.org/10.1016/j.carbpol.2014.12.076.
[76] T. Martins, A.A.R. Oliveira, A.C. Oliveira, T.P. Boaventura, B.R. Barrioni, E.S. Costa-Júnior, M.M. Pereira, Novel 3D composites with highly flexible behavior based on chitosan and bioactive glass for biomedical applications, Mater Chem Phys, 189 (2017) 1-11, https://doi.org/10.1016/j.matchemphys.2016.12.012.
[77] M. Peter, N.S. Binulal, S. Soumya, S.V. Nair, T. Furuike, H. Tamura, R. Jayakumar, Nanocomposite scaffolds of bioactive glass ceramic nanoparticles disseminated chitosan matrix for tissue engineering applications, Carbohydr Polym, 79 (2010) 284-289, http://dx.doi.org/10.1016/j.carbpol.2009.08.001.
[78] E.M.F. Lemos, P.S.O. Patrício, M.M. Pereira, 3D nanocomposite chitosan/bioactive glass scaffolds obtained using two different routes: An evaluation of the porous structure and mechanical properties, Quím Nova, 39 (2016) 462-466, https://doi.org/10.5935/0100-4042.20160047.
[79] J.A. Roether, J.E. Gough, A.R. Boccaccini, L.L. Hench, V. Maquet, R. Jérôme, Novel bioresorbable and bioactive composites based on bioactive glass and polylactide foams for bone tissue engineering, J Mater Sci Mater Med, 13 (2002) 1207-1214, https://doi.org/10.1023/a:1021166726914.
[80] E.M. Valliant, J.R. Jones, Softening bioactive glass for bone regeneration: sol–gel hybrid materials, Soft Matter, 7 (2011) 5083-5095, https://doi.org/10.1039/c0sm01348j.
[81] N. Hild, P.N. Tawakoli, J.G. Halter, B. Sauer, W. Buchalla, W.J. Stark, D. Mohn, pH-dependent antibacterial effects on oral microorganisms through pure PLGA implants and composites with nanosized bioactive glass, Acta Biomater, 9 (2013) 9118-9125, https://doi.org/10.1016/j.actbio.2013.06.030.

[82] A. Ardeshirylajimi, S. Farhadian, F.J. Adegani, S. Mirzaei, M.S. Zomorrod, L. Langroudi, A. Doostmohammadi, E. Seyedjafari, M. Soleimani, Enhanced osteoconductivity of polyethersulphone nanofibres loaded with bioactive glass nanoparticles in in vitro and in vivo models, Cell Prolif, 48 (2015) 455-464, https://doi.org/10.1111/cpr.12198.
[83] D. Wang, H. Lin, J. Jiang, Q. Jin, L. Li, Y. Dong, F. Qu, Fabrication of long-acting drug release property of hierarchical porous bioglasses/polylactic acid fibre scaffolds for bone tissue engineering, IET Nanobiotechnol, 9 (2015) 58-65, https://doi.org/10.1049/iet-nbt.2013.0011.
[84] J. Ren, K.A. Blackwood, A. Doustgani, P.P. Poh, R. Steck, M.M. Stevens, M.A. Woodruff, Melt-electrospun polycaprolactone strontium-substituted bioactive glass scaffolds for bone regeneration, J Biomed Mater Res A, 102 (2014) 3140-3153, https://doi.org/10.1002/jbm.a.34985.
[85] K. Ahtiainen, L. Sippola, M. Nurminen, B. Mannerstrom, S. Haimi, R. Suuronen, J. Hyttinen, T. Ylikomi, M. Kellomaki, S. Miettinen, Effects of chitosan and bioactive glass modifications of knitted and rolled polylactide-based 96/4 L/D scaffolds on chondrogenic differentiation of adipose stem cells, J Tissue Eng Regen Med, 9 (2015) 55-65, https://doi.org/10.1002/term.1614.
[86] A.R. Boccaccini, M. Erol, W.J. Stark, D. Mohn, Z. Hong, J.F. Mano, Polymer/bioactive glass nanocomposites for biomedical applications: A review, Compos Sci Technol, 70 (2010) 1764-1776, https://doi.org/10.1016/j.compscitech.2010.06.002.
[87] M.E. Santocildes-Romero, R.L. Goodchild, P.V. Hatton, A. Crawford, I.M. Reaney, C.A. Miller, Preparation of composite electrospun membranes containing strontium-substituted bioactive glasses for bone tissue regeneration, Macromol Mater Eng, 301 (2016) 972-981, https://doi.org/10.1002/mame.201600018.
[88] Y. Ding, W. Li, T. Muller, D.W. Schubert, A.R. Boccaccini, Q. Yao, J.A. Roether, Electrospun polyhydroxybutyrate/poly(epsilon-caprolactone)/58S sol-gel bioactive glass hybrid scaffolds with highly improved osteogenic potential for bone tissue engineering, ACS Appl Mater Interfaces, 8 (2016) 17098-17108, https://doi.org/10.1021/acsami.6b03997.
[89] M.R. Foroughi, S. Karbasi, M. Khoroushi, A.A. Khademi, Polyhydroxybutyrate/chitosan/bioglass nanocomposite as a novel electrospun scaffold: Fabrication and characterization, J Porous Mater, 24 (2017) 1447-1460, https://doi.org/10.1007/s10934-017-0385-2.
[90] S. Shahabi, Y. Rezaei, F. Moztarzadeh, F. Najafi, In vitro degradation and bioactivity of poly(propylene fumarate)/bioactive glass sintered microsphere scaffolds for bone tissue engineering, Sci Eng Compos Mater, 23 (2016) 245-256, https://doi.org/10.1515/secm-2014-0116.
[91] W. Huang, X. Li, X. Shi, C. Lai, Microsphere based scaffolds for bone regenerative applications, Biomater Sci, 2 (2014) 1145-1153, https://doi.org/10.1039/c4bm00161c.

[92] F.N. Alaribe, S.L. Manoto, S.C.K.M. Motaung, Scaffolds from biomaterials: Advantages and limitations in bone and tissue engineering, Biologia, 71 (2016) 353-366, https://doi.org/10.1515/biolog-2016-0056.
[93] X. Shi, K. Su, R.R. Varshney, Y. Wang, D.A. Wang, Sintered microsphere scaffolds for controlled release and tissue engineering, Pharm Res, 28 (2011) 1224-1228, https://doi.org/10.1007/s11095-010-0359-4.
[94] X. Shi, Y. Wang, L. Ren, C. Lai, Y. Gong, D.A. Wang, A novel hydrophilic poly(lactide-co-glycolide)/lecithin hybrid microspheres sintered scaffold for bone repair, J Biomed Mater Res A, 92 (2010) 963-972, https://doi.org/10.1002/jbm.a.32423.
[95] L. Liverani, J.A. Roether, P. Nooeaid, M. Trombetta, D.W. Schubert, A.R. Boccaccini, Simple fabrication technique for multilayered stratified composite scaffolds suitable for interface tissue engineering, Mater Sci Eng C, 557 (2012) 54-58, https://doi.org/10.1016/j.msea.2012.05.104.
[96] Q. Yao, P. Nooeaid, R. Detsch, J.A. Roether, Y. Dong, O.M. Goudouri, D.W. Schubert, A.R. Boccaccini, Bioglass®/chitosan-polycaprolactone bilayered composite scaffolds intended for osteochondral tissue engineering, J Biomed Mater Res A, 102 (2014) 4510-4518, https://doi.org/10.1002/jbm.a.35125.
[97] P. Balasubramanian, A.R. Boccaccini, Bilayered bioactive glass scaffolds incorporating fibrous morphology by flock technology, Mater Lett, 158 (2015) 313-316, https://doi.org/10.1016/j.matlet.2015.06.036.
[98] C.M. Piard, Y. Chen, J.P. Fisher, Cell-laden 3D printed scaffolds for bone tissue engineering, Clin Rev Bone Miner Metab, 13 (2015) 245-255, https://doi.org/10.1007/s12018-015-9198-5.
[99] W. Helen, J.E. Gough, Cell viability, proliferation and extracellular matrix production of human annulus fibrosus cells cultured within PDLLA/bioglass composite foam scaffolds in vitro, Acta Biomater, 4 (2008) 230-243, https://doi.org/10.1016/j.actbio.2007.09.010.
[100] S.J. Florczyk, M. Leung, S. Jana, Z. Li, N. Bhattarai, J.I. Huang, R.A. Hopper, M. Zhang, Enhanced bone tissue formation by alginate gel-assisted cell seeding in porous ceramic scaffolds and sustained release of growth factor, J Biomed Mater Res A, 100A (2012) 3408-3415, https://doi.org/10.1002/jbm.a.34288.
[101] F. Westhauser, C. Weis, M. Prokscha, L.A. Bittrich, W. Li, K. Xiao, U. Kneser, H.-U. Kauczor, G. Schmidmaier, A.R. Boccaccini, A. Moghaddam, Three-dimensional polymer coated 45S5-type bioactive glass scaffolds seeded with human mesenchymal stem cells show bone formation in vivo, J Mater Sci Mater Med, 27 (2016) 119-125, https://doi.org/10.1007/s10856-016-5732-3.

[102] C. Chen, H. Li, J. Pan, Z. Yan, Z. Yao, W. Fan, C. Guo, Biodegradable composite scaffolds of bioactive glass/chitosan/carboxymethyl cellulose for hemostatic and bone regeneration, Biotechnol Lett, 37 (2015) 457-465, https://doi.org/10.1007/s10529-014-1697-9.
[103] X.B. Yang, D. Webb, J. Blaker, A.R. Boccaccini, V. Maquet, C. Cooper, R.O.C. Oreffo, Evaluation of human bone marrow stromal cell growth on biodegradable polymer/Bioglass® composites, Biochem Biophys Res Commun, 342 (2006) 1098-1107, https://doi.org/10.1016/j.bbrc.2006.02.021.
[104] J. Filipowska, K. Cholewa-Kowalska, J. Wieczorek, D. Semik, Z. Dabrowski, M. Laczka, A.M. Osyczka, Ectopic bone formation by gel-derived bioactive glass-poly-L-lactide-co-glycolide composites in a rabbit muscle model, Biomed Mater, 12 (2017) 015015, https://doi.org/10.1088/1748-605X/aa4eb7.
[105] R.M. Day, V. Maquet, A.R. Boccaccini, R. Jerome, A. Forbes, In vitro and in vivo analysis of macroporous biodegradable poly(D,L-lactide-co-glycolide) scaffolds containing bioactive glass, J Biomed Mater Res A, 75 (2005) 778-787, https://doi.org/10.1002/jbm.a.30433.
[106] A.N. Renth, M.S. Detamore, Leveraging "raw materials" as building blocks and bioactive signals in regenerative medicine, Tissue Eng Part B Rev, 18 (2012) 341-362, https://doi.org/10.1089/ten.TEB.2012.0080.
[107] W. Lu, K. Ji, J. Kirkham, Y. Yan, A.R. Boccaccini, M. Kellett, Y. Jin, X.B. Yang, Bone tissue engineering by using a combination of polymer/bioglass composites with human adipose-derived stem cells, Cell Tissue Res, 356 (2014) 97-107, https://doi.org/10.1007/s00441-013-1770-z.
[108] A.K. Shakya, R. Holmdahl, K.S. Nandakumar, A. Kumar, Polymeric cryogels are biocompatible, and their biodegradation is independent of oxidative radicals, J Biomed Mater Res A, 102 (2014) 3409-3418, https://doi.org/10.1002/jbm.a.35013.
[109] D.B. Raina, H. Isaksson, A.K. Teotia, L. Lidgren, M. Tagil, A. Kumar, Biocomposite macroporous cryogels as potential carrier scaffolds for bone active agents augmenting bone regeneration, J Control Release, 235 (2016) 365-378, https://doi.org/10.1016/j.jconrel.2016.05.061.
[110] A.M. Getgood, S.J. Kew, R. Brooks, H. Aberman, T. Simon, A.K. Lynn, N. Rushton, Evaluation of early-stage osteochondral defect repair using a biphasic scaffold based on a collagen-glycosaminoglycan biopolymer in a caprine model, Knee, 19 (2012) 422-430, https://doi.org/10.1016/j.knee.2011.03.011.
[111] D. Albano, N. Martinelli, A. Bianchi, C. Messina, F. Malerba, L.M. Sconfienza, Clinical and imaging outcome of osteochondral lesions of the talus treated using autologous matrix-induced chondrogenesis technique with a biomimetic scaffold, BMC Musculoskelet Disord, 18 (2017) 306-312, https://doi.org/10.1186/s12891-017-1679-x.

[112] E. Kon, G. Filardo, F. Perdisa, G. Venieri, M. Marcacci, Clinical results of multilayered biomaterials for osteochondral regeneration, J Exp Orthop, 1 (2014) 10-17, https://doi.org/10.1186/s40634-014-0010-0.
[113] Collagen solutions, *Positive Eight-Year Results of ChondroMimetic® Cartilage Repair Clinical Study*. 2018 [cited 2018 9 July]; Available from: http://ir.collagensolutions.com/content/news/2018/210218.asp.
[114] F. Perdisa, G. Filardo, B. Di Matteo, A. Di Martino, M. Marcacci, Biological knee reconstruction: a case report of an Olympic athlete, Eur Rev Med Pharmaco, 18 (2014) 76-80, http://europepmc.org/abstract/MED/24825047.
[115] C.N. Cornell, J.M. Lane, M. Chapman, R. Merkow, D. Seligson, S. Henry, R. Gustilo, K. Vincent, Multicenter trial of Collagraft as bone graft substitute, Journal of orthopaedic trauma, 5 (1991) 1-8, https://doi.org/10.1097/00005131-199105020-00018.
[116] T. Lerner, V. Bullmann, T.L. Schulte, M. Schneider, U. Liljenqvist, A level-1 pilot study to evaluate of ultraporous β-tricalcium phosphate as a graft extender in the posterior correction of adolescent idiopathic scoliosis, European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society, 18 (2009) 170-179, https://doi.org/10.1007/s00586-008-0844-1.
[117] D. Neen, D. Noyes, M. Shaw, S. Gwilym, N. Fairlie, N. Birch, Healos and bone marrow aspirate used for lumbar spine fusion: a case controlled study comparing healos with autograft, Spine, 31 (2006) E636-640, https://doi.org/10.1097/01.brs.0000232028.97590.12.
[118] J.P. McCoy Jr, W. Schade, R.J. Siegle, E.E. Vanderveen, C.B. Zachary, T.P. Waldinger, N.A. Swanson, Immune responses to bovine collagen implants. Significance of pretreatment serology, Journal of the American Academy of Dermatology, 16 (1987) 955-960, https://doi.org/10.1016/S0190-9622(87)70120-0.
[119] T.T. Roberts, A.J. Rosenbaum, Bone grafts, bone substitutes and orthobiologics: The bridge between basic science and clinical advancements in fracture healing, Organogenesis, 8 (2012) 114-124, https://doi.org/10.4161/org.23306.
[120] S. Eqtesadi, A. Motealleh, A. Pajares, F. Guiberteau, P. Miranda, Improving mechanical properties of 13–93 bioactive glass robocast scaffold by poly (lactic acid) and poly (ε-caprolactone) melt infiltration, J Non-Cryst Solids, 432 (2016) 111-119, https://doi.org/10.1016/j.jnoncrysol.2015.02.025.
[121] C. Vichery, J.-M. Nedelec, Bioactive glass nanoparticles: From synthesis to materials design for biomedical applications, Materials, 9 (2016) 288-305, https://doi.org/10.3390/ma9040288.
[122] J. Jones, A. Clare, Bio-Glasses : An Introduction, John Wiley & Sons, Incorporated, 2012.

[123] R. Ravarian, M. Craft, F. Dehghani, Enhancing the biological activity of chitosan and controlling the degradation by nanoscale interaction with bioglass, J Biomed Mater Res A, 103 (2015) 2898-2908, https://doi.org/10.1002/jbm.a.35423.
[124] Y. Shirosaki, Preparation of organic-inorganic hybrids with silicate network for the medical applications, J Ceram Soc Jpn, 120 (2012) 555-559, https://doi.org/10.2109/jcersj2.120.555.
[125] J. Lao, X. Dieudonné, F. Fayon, V. Montouillout, E. Jallot, Bioactive glass–gelatin hybrids: Building scaffolds with enhanced calcium incorporation and controlled porosity for bone regeneration, J Mater Chem B, 4 (2016) 2486-2497, https://doi.org/10.1039/c5tb02345a.
[126] A. Liu, Z. Hong, X. Zhuang, X. Chen, Y. Cui, Y. Liu, X. Jing, Surface modification of bioactive glass nanoparticles and the mechanical and biological properties of poly(L-lactide) composites, Acta Biomater, 4 (2008) 1005-1015, https://doi.org/10.1016/j.actbio.2008.02.013.
[127] K. Zhang, Y. Wang, M.A. Hillmyer, L.F. Francis, Processing and properties of porous poly(l-lactide)/bioactive glass composites, Biomaterials, 25 (2004) 2489-2500, https://doi.org/10.1016/j.biomaterials.2003.09.033.
[128] V.C. Costa, H.S. Costa, W.L. Vasconcelos, M.d.M. Pereira, R.L. Oréfice, H.S. Mansur, Preparation of hybrid biomaterials for bone tissue engineering, Mat Res, 10 (2007) 21-26, http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-14392007000100006&nrm=iso.
[129] A.R.P.d. Silva, T.L. Macedo, D.J. Coletta, S. Feldman, M.d.M. Pereira, Synthesis, characterization and cytotoxicity of chitosan/polyvinyl alcohol/bioactive glass hybrid scaffolds obtained by lyophilization, Materia Brazil, 21 (2016) 964-973, http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1517-70762016000400964&nrm=iso.
[130] H.S. Mansur, H.S. Costa, Nanostructured poly(vinyl alcohol)/bioactive glass and poly(vinyl alcohol)/chitosan/bioactive glass hybrid scaffolds for biomedical applications, Chem Eng J, 137 (2008) 72-83, https://doi.org/10.1016/j.cej.2007.09.036.
[131] R.C.F. Cheung, T.B. Ng, J.H. Wong, W.Y. Chan, Chitosan: An update on potential biomedical and pharmaceutical applications, Mar Drugs, 13 (2015) 5156-5186, http://dx.doi.org/10.3390/md13085156.
[132] M.N.V. Ravi Kumar, A review of chitin and chitosan applications, React Funct Polym, 46 (2000) 1-27, http://dx.doi.org/10.1016/S1381-5148(00)00038-9.
[133] R. LogithKumar, A. KeshavNarayan, S. Dhivya, A. Chawla, S. Saravanan, N. Selvamurugan, A review of chitosan and its derivatives in bone tissue engineering, Carbohydr Polym, 151 (2016) 172-188, http://dx.doi.org/10.1016/j.carbpol.2016.05.049.
[134] N. Bhattarai, J. Gunn, M. Zhang, Chitosan-based hydrogels for controlled, localized drug delivery, Adv Drug Deliv Rev, 62 (2010) 83-99, https://doi.org/10.1016/j.addr.2009.07.019.

[135] W.E. Hennink, C.F. van Nostrum, Novel crosslinking methods to design hydrogels, Adv Drug Deliv Rev, 64 (2012) 223-236, https://doi.org/10.1016/j.addr.2012.09.009.
[136] E.M. Ahmed, Hydrogel: Preparation, characterization, and applications: A review, J Adv Res, 6 (2015) 105-121, https://doi.org/10.1016/j.jare.2013.07.006.
[137] L. Gao, H. Gan, Z. Meng, R. Gu, Z. Wu, L. Zhang, X. Zhu, W. Sun, J. Li, Y. Zheng, G. Dou, Effects of genipin cross-linking of chitosan hydrogels on cellular adhesion and viability, Colloids Surf B Biointerfaces, 117 (2014) 398-405, https://doi.org/10.1016/j.colsurfb.2014.03.002.
[138] Y.J. Hwang, J. Larsen, T.B. Krasieva, J.G. Lyubovitsky, Effect of genipin crosslinking on the optical spectral properties and structures of collagen hydrogels, ACS Appl Mater Interfaces, 3 (2011) 2579-2584, https://doi.org/10.1021/am200416h.
[139] S. Matcham, K. Novakovic, Fluorescence imaging in genipin crosslinked chitosan–poly(vinyl pyrrolidone) hydrogels, Polymers, 8 (2016) 385-394, https://doi.org/10.3390/polym8110385.
[140] G.A. Hurst, K. Novakovic, A facile in situ morphological characterization of smart genipin-crosslinked chitosan–poly(vinyl pyrrolidone) hydrogels, J Mater Res, 28 (2013) 2401-2408, https://doi.org/10.1557/jmr.2013.134.
[141] A. Oryan, A. Kamali, A. Moshiri, H. Baharvand, H. Daemi, Chemical crosslinking of biopolymeric scaffolds: Current knowledge and future directions of crosslinked engineered bone scaffolds, Int J Biol Macromol, 107 (2018) 678-688, https://doi.org/10.1016/j.ijbiomac.2017.08.184.
[142] K.Y. Chung, M.A. Birch, K. Novakovic, Genipin-crosslinked chitosan hydrogels as scaffolds for mammalian cell growth, Int J Adv Res Sci Eng Technol, 6 (2018) 37-44, http://www.iraj.in/journal/journal_file/journal_pdf/6-439-152265063937-44.pdf.
[143] W. Zhang, G. Ren, H. Xu, J. Zhang, H. Liu, S. Mu, X. Cai, T. Wu, Genipin cross-linked chitosan hydrogel for the controlled release of tetracycline with controlled release property, lower cytotoxicity, and long-term bioactivity, Journal of Polym Res, 23 (2016) 156-164, https://doi.org/10.1007/s10965-016-1059-5.
[144] F. Ahmadi, Z. Oveisi, S.M. Samani, Z. Amoozgar, Chitosan based hydrogels: Characteristics and pharmaceutical applications, Res Pharm Sci, 10 (2015) 1-16, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4578208/.
[145] R.A.A. Muzzarelli, Genipin-crosslinked chitosan hydrogels as biomedical and pharmaceutical aids, Carbohydr Polym, 77 (2009) 1-9, http://dx.doi.org/10.1016/j.carbpol.2009.01.016.
[146] H. Chen, W. Ouyang, B. Lawuyi, S. Prakash, Genipin cross-linked alginate-chitosan microcapsules: membrane characterization and optimization of cross-linking reaction, Biomacromolecules, 7 (2006) 2091-2098, https://doi.org/10.1021/bm050862y.

[147] F.-L. Mi, H.-W. Sung, S.-S. Shyu, Release of indomethacin from a novel chitosan microsphere prepared by a naturally occurring crosslinker: Examination of crosslinking and polycation–anionic drug interaction, J Appl Polym Sci, 81 (2001) 1700-1711, https://doi.org/10.1002/app.1602.
[148] J. Nie, Z. Wang, Q. Hu, Difference between chitosan hydrogels via alkaline and acidic solvent systems, Sci Rep, 6 (2016) 36053-36060, https://doi.org/10.1038/srep36053 http://www.nature.com/articles/srep36053#supplementary-information.
[149] C.J. Nwosu, G.A. Hurst, K. Novakovic, Genipin cross-linked chitosan-polyvinylpyrrolidone hydrogels: Influence of composition and postsynthesis treatment on pH responsive behaviour, Adv Mater Sci Eng, 2015 (2015) 1-10, https://doi.org/10.1155/2015/621289.
[150] S. Dimida, C. Demitri, V.M. De Benedictis, F. Scalera, F. Gervaso, A. Sannino, Genipin-cross-linked chitosan-based hydrogels: Reaction kinetics and structure-related characteristics, J Appl Polym Sci, 132 (2015) 42256-42263, https://doi.org/10.1002/app.42256.